The influence of larval diet and microsporidian infection on life history traits of the forest tent caterpillar, *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae).

by

Flavio Preti

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Abstract

The forest tent caterpillar (FTC), *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae), is an important ecological disturbance factor of deciduous hardwood trees in North America. Disturbance is greater during outbreaks; a cyclical phenomenon in which large numbers of FTC larvae cause extensive defoliation to host trees. Outbreaks are linked to density-induced alterations of food quality and/or quantity, and increased entomopathogen abundance. Variation in FTC larval diet is known to affect life history traits and resource allocation to flight and reproduction in adult moths. Furthermore, differences in phytochemistry among host plants can alter resistance to entomopathogens. In this thesis, I use different approaches to investigate the tritrophic interaction of larval food source, FTC, and the microsporidian parasite of FTC, *Nosema disstriae* (Microsporida: Nosematidae). First, I examined the effects of qualitative and quantitative manipulations of an artificial larval diet and microsporidian infection by direct inoculation of FTC on the development time, cocoon production and weight, and alterations in allometric relationship between wing area (mm^2) and body weight (mg). Quantitative food manipulation simulated density-induced food stress associated with high population density. Qualitative manipulation of larval diet included supplementation of artificial diet with 1% lyophilized foliage of trembling aspen, *Populus tremuloides* Michx (Malpighiales: Salicaceae), the main host of FTC in Canada. Microsporidian infection was administrated via inoculation at third instar larva. In a second experiment, I focused on the effects of larval host and natural infection by microsporidia of FTC on adult body size (wing area) and fitness. Forest tent caterpillar larvae were reared on two host species, trembling aspen and sugar maple, *Acer saccharum* Marshall (Sapindales: Sapindaceae). Results from my studies suggest larval diet does not interact with microsporidian infection to affect adult life history traits and resource allocation in FTC, although diet and infection alone influenced life history traits and resource allocation. Microsporidian infection inhibited cocoon production, likely as result of heavy infection in the silk glands. Severe microsporidian infection was associated with reduced wing area and the loss of

allometric relationship between wing area and body weight, which implies infection by microsporidia can alter FTC evolutionary trade-offs between reproduction and dispersal. In contrast to other insects, microsporidian infection was not associated with wing malformation in FTC. Addition of lyophilized trembling aspen foliage to the larval diet led to increased wing malformation, longer development time, and loss of the allometric relationship between wing area and body weight. These effects are likely the result of aspen secondary metabolites in the diet. Partial starvation resulted in increased development time, reduced adult size, and loss of the allometric relationship between wing area and body weight, suggesting density-related nutritional stress may affect dispersal by flight in FTC. The larval host study provides further evidence that trembling aspen is a more suitable host for FTC than sugar maple. My studies are the first to assess the potential interaction between larval diet and microsporidian infection in FTC. The results suggest host phytochemistry and density-induced resource limitation do not interact with microsporidian infection in FTC, but they likely work in an additive fashion to influence life history traits that contribute to population cycling in this species.

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Table of Contents

Chapter 1 – General Introduction 1
Outbreaking forest lepidoptera 1
Malacosoma disstria Hübner 4
Host Use 6
Natural Control 9
Microsporidia 11
Diet-Disease Interactions 14
Thesis Objective 15
References 16
Chapter 2 – Effects of larval diet quality and quantity and microsporidian infection on life history traits of the forest tent caterpillar, *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae). 37
Abstract 37
Introduction 38
Materials and Methods 41
Caterpillar rearing 41
Preparation of artificial diet 43
Feeding Regimes 43
Infection levels 43
Microsporidian infection assessment 44
Trait measurements 44
Statistical analyses 45
Results 49
Cocoon Production 49
Cocoon Weight 49
Development Time 50
Adult Weight 51
Wing Area 51
Allometric relationship (mg/mm^2) 52
Infection 53
Discussion 53
Tables 62
Figures 64
References 79

Chapter 3 – Effects of larval host and microsporidian infection on wing area of the forest tent caterpillar, *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae). 94
Abstract 94
Introduction 94
Materials and Methods 97
Caterpillar rearing 97
Wing Area Measurements 99
Microsporidia Infection Assessment 100
Statistical Analyses 2014 Experiment 100
Statistical Analysis 2018 Experiment 102
Results 103
Wing Area (2014) 103
Infection (2014) 104
Wing Area (2018) 105
Malformed Wings (2018) 105
Infection (2018) 106
Discussion 106
Tables 113
Figures 116
References 126
Chapter 4 – Conclusions 135
References 141
Bibliography 148
Appendix A 181
Appendix B 182
Appendix C 183
Appendix D 184
Appendix E 186
Appendix F 187

List of Tables

Table 2-1. List of models used for statistical analyses. Diet (SD = standard diet, SDA = standard diet + trembling aspen), Feeding Regime (F = full regime, S = starved), Origin (Alberta, Ontario), Sex (M = Males, F = Females), and Infection Load (None: 0 spores, Low: 1-100 spores, High: 101+ spores count per 10 fields of view at 400x magnification). 62
Table 2-2 Population sizes (N) used for each analysis. Diet (SD = standard diet, SDA = standard diet + trembling aspen), Feeding Regime (F = full regime, S = partially starved), Infection Load (N: 0 spores, Low: 1-100 spores, High: 101+ spores count per 10 fields of view at 400x magnification), and Origin (Alberta, Ontario). 63
Table 3-1. List of models used for statistical analyses of 2014 data. Diet (Diet = artificial standard diet, Aspen = fresh trembling aspen foliage), Origin (AB = Alberta, OD = Ontario – Dryden, OK = Ontario – Kenora), Sex (M = Males, F = Females), Infection (N = Uninfected, Y = Infected), and Infection Load (None: 0 spores, Low: 1-100 spores, High: 101+ spores count per 10 fields of view at 400x magnification). 113
Table 3-2. Population sizes (N) used for each analysis divided by Diet (Diet = artificial standard diet, Aspen = fresh trembling aspen foliage), Infection (N = Uninfected, Low: 1-100 spores, High: 101+ spores count per 10 fields of view at 400x magnification), and Origin (AB = Alberta, OD = Ontario – Dryden, OK = Ontario – Kenora). 114
Table 3-3. List of models used for statistical analyses of 2018 data. Diet (SD = standard diet, FA = fresh trembling aspen foliage, FM = fresh sugar maple foliage), Origin (Alberta, Ontario), Sex (M = Males, F = Females), Infection (N = Uninfected, Y = Infected), and Infection Load (None: 0 spores, Low: 1-100 spores, High: 101+ spores count per 10 fields of view at 400x magnification). 114
Table 3-4. Population sizes (N) used for each analysis divided by Diet (SD = standard diet, FA = fresh trembling aspen foliage, FM = fresh sugar maple foliage) and Infection (N = Uninfected, Low: 1-100 spores, High: 101+ spores count per 10 fields of view at 400x magnification). 115

List of Figures

FIGURE 2-1. EXPERIMENTAL DESIGN OF THE TREATMENTS TESTED. BASED ON ORIGIN: ALBERTA & ONTARIO, DIET: SD = STANDARD DIET, SDA = STANDARD DIET + 1% LYOPHILIZED TREMBLING ASPEN FOLIAGE, FEEDING REGIME: F = FULL REGIME, S = PARTIALLY STARVED, AND INFECTION: N = UNINFECTED, Y = INFECTED. 64
FIGURE 2-2. PYRAMID PLOTS OF COCOON PRODUCTION (N = NO COCOON, Y = COCOON PRESENT) OF ALL FTC THAT REACHED PUPATION. (A) DIET: SD = STANDARD DIET, SDA = STANDARD DIET + 1% LYOPHILIZED TREMBLING ASPEN FOLIAGE, (B) FEEDING REGIME: F = FULL REGIME, S = PARTIALLY STARVED, (C) ORIGIN: ALBERTA & ONTARIO, AND (D) INFECTION LOAD: NONE = 0 SPORES, LOW = 1 < SPORES < 100, HIGH = 100+ SPORES PER 10 FIELDS OF VIEW AT 400X MAGNIFICATION. 65
FIGURE 2-3. PYRAMID PLOTS OF COCOON PRODUCTION (N = NO COCOON, Y = COCOON PRESENT) OF ALL FTC THAT REACHED PUPATION BY LARVAL INFECTION STATUS: N = UNINFECTED, Y = INFECTED BY INOCULATION, YY = INFECTED NATURALLY. 66
FIGURE 2-4. BOXPLOTS OF COCOON WEIGHT (MG) OF FEMALE FTC BY (A) DIET: SD = STANDARD DIET, SDA = STANDARD DIET + TREMBLING ASPEN FOLIAGE, (B) ORIGIN: ALBERTA & ONTARIO, AND (C) INFECTION LOAD: NONE = 0 SPORES, LOW = 1 < SPORES < 100, HIGH = 100+ SPORES PER 10 FIELDS OF VIEW AT 400X MAGNIFICATION. THE MIDLINE OF EACH BOX REPRESENTS THE MEDIAN, WHEREAS THE BOTTOM AND TOP OF EACH BOX INDICATES THE FIRST AND THIRD QUARTILES, RESPECTIVELY. THE WHISKERS EXTENDING FROM EACH BOX INDICATE THE MAXIMUM AND MINIMUM VALUES AND THE BLACK DOTS ARE OUTLIERS. BOXPLOTS MARKED WITH DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (T TEST, P < 0.05). 67
FIGURE 2-5. BOXPLOTS OF COCOON WEIGHT (MG) OF MALE FTC BY (A) DIET: SD = STANDARD DIET, SDA = STANDARD DIET + TREMBLING ASPEN FOLIAGE, (B) FEEDING REGIME: F = FULL REGIME, S = STARVED, (C) ORIGIN: ALBERTA & ONTARIO, AND (D) INFECTION LOAD: NONE = 0 SPORES, LOW = 1 < SPORES < 100, HIGH = 100+ SPORES PER 10 FIELDS OF VIEW AT 400X MAGNIFICATION. THE MIDLINE OF EACH BOX REPRESENTS THE MEDIAN, WHEREAS THE BOTTOM AND TOP OF EACH BOX INDICATES THE FIRST AND THIRD QUARTILES, RESPECTIVELY. THE WHISKERS EXTENDING FROM EACH BOX INDICATE THE MAXIMUM AND MINIMUM VALUES AND THE BLACK DOTS ARE OUTLIERS. BOXPLOTS MARKED WITH DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (T TEST, P < 0.05). 68
FIGURE 2-6. BOXPLOTS OF NUMBER OF DAYS REQUIRED FROM EGG HATCH FTC TO ADULT ECLOSION BY (A) DIET: SD = STANDARD DIET, SDA = STANDARD DIET + TREMBLING ASPEN FOLIAGE, (B) FEEDING REGIME: F = FULL REGIME, S = STARVED, (C) INFECTION LOAD: NONE = 0 SPORES, LOW = 1 < SPORES < 100, HIGH = 100+ SPORES PER 10 FIELDS OF VIEW AT 400X MAGNIFICATION, (D) ORIGIN: ALBERTA & ONTARIO, AND (E) SEX: F = FEMALES, M = MALES. THE MIDLINE OF EACH BOX REPRESENTS THE MEDIAN, WHEREAS THE BOTTOM AND TOP OF EACH BOX INDICATES THE FIRST AND THIRD QUARTILES, RESPECTIVELY. THE WHISKERS EXTENDING FROM EACH BOX INDICATE THE MAXIMUM AND MINIMUM VALUES AND THE BLACK DOTS ARE OUTLIERS. BOXPLOTS MARKED WITH DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (T TEST, P < 0.05). 69
FIGURE 2-7. BOXPLOTS OF NUMBER OF DAYS REQUIRED FOR DEVELOPMENT OF FTC FROM EGG HATCH TO PUPATION BY (A) DIET: SD = STANDARD DIET, SDA = STANDARD DIET + TREMBLING ASPEN FOLIAGE, (B) STARVATION: F = FULL REGIME, S = STARVED, (C) INFECTION LOAD: NONE = 0 SPORES, LOW = 1 < SPORES < 100, HIGH = 100+ SPORES PER 10 FIELDS OF VIEW AT 400X MAGNIFICATION, AND (D) ORIGIN: ALBERTA & ONTARIO. THE MIDLINE OF EACH BOX REPRESENTS THE MEDIAN, WHEREAS THE BOTTOM AND TOP OF EACH BOX INDICATES THE FIRST AND THIRD QUARTILES, RESPECTIVELY. THE WHISKERS EXTENDING FROM EACH BOX INDICATE THE MAXIMUM AND MINIMUM VALUES AND THE BLACK DOTS ARE OUTLIERS. BOXPLOTS MARKED WITH DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (T TEST, P < 0.05). 70

FIGURE 2-8. BOXPLOTS OF NUMBER OF DAYS REQUIRED BY FTC FOR PUPAL DEVELOPMENT BY (A) DIET: SD = STANDARD DIET, SDA = STANDARD DIET + TREMBLING ASPEN FOLIAGE, (B) FEEDING REGIME: F = FULL REGIME, S = STARVED, (C) INFECTION LOAD: NONE = 0 SPORES, LOW = 1 < SPORES < 100, HIGH = 100+ SPORES PER 10 FIELDS OF VIEW AT 400X MAGNIFICATION, (D) ORIGIN: ALBERTA & ONTARIO, AND (E) SEX: F = FEMALES, M = MALES. THE MIDLINE OF EACH BOX REPRESENTS THE MEDIAN, WHEREAS THE BOTTOM AND TOP OF EACH BOX INDICATES THE FIRST AND THIRD QUARTILES, RESPECTIVELY. THE WHISKERS EXTENDING FROM EACH BOX INDICATE THE MAXIMUM AND MINIMUM VALUES AND THE BLACK DOTS ARE OUTLIERS. BOXPLOTS MARKED WITH DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (T TEST, $P < 0.05$). 71
FIGURE 2-9. BOXPLOTS OF MOTH WEIGHT (MG) OF FEMALE FTC BY (A) DIET: SD = STANDARD DIET, SDA = STANDARD DIET + TREMBLING ASPEN FOLIAGE, (B) INFECTION LOAD: NONE = 0 SPORES, LOW = 1 < SPORES < 100, HIGH = 100+ SPORES PER 10 FIELDS OF VIEW AT 400X MAGNIFICATION, AND (C) ORIGIN: ALBERTA & ONTARIO. THE MIDLINE OF EACH BOX REPRESENTS THE MEDIAN, WHEREAS THE BOTTOM AND TOP OF EACH BOX INDICATES THE FIRST AND THIRD QUARTILES, RESPECTIVELY. THE WHISKERS EXTENDING FROM EACH BOX INDICATE THE MAXIMUM AND MINIMUM VALUES AND THE BLACK DOTS ARE OUTLIERS. BOXPLOTS MARKED WITH DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (T TEST, $P < 0.05$). 72
FIGURE 2-10.BOXPLOTS OF MOTH WEIGHT (MG) OF MALE FTC BY (A) DIET: SD = STANDARD DIET, SDA = STANDARD DIET + TREMBLING ASPEN FOLIAGE, (B) FEEDING REGIME: F = FULL REGIME, S = STARVED, (C) INFECTION LOAD: NONE = 0 SPORES, LOW = 1 < SPORES < 100, HIGH = 100+ SPORES PER 10 FIELDS OF VIEW AT 400X MAGNIFICATION, AND (D) ORIGIN: ALBERTA & ONTARIO. THE MIDLINE OF EACH BOX REPRESENTS THE MEDIAN, WHEREAS THE BOTTOM AND TOP OF EACH BOX INDICATES THE FIRST AND THIRD QUARTILES, RESPECTIVELY. THE WHISKERS EXTENDING FROM EACH BOX INDICATE THE MAXIMUM AND MINIMUM VALUES AND THE BLACK DOTS ARE OUTLIERS. BOXPLOTS MARKED WITH DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (T TEST, $P < 0.05$). 73
FIGURE 2-11. BOXPLOTS OF WING AREA (MM^2) OF FEMALE FTC BY (A) DIET: SD = STANDARD DIET, SDA = STANDARD DIET + TREMBLING ASPEN FOLIAGE, (B) INFECTION: UNINFECTED & INFECTED, AND (C) ORIGIN: ALBERTA & ONTARIO. THE MIDLINE OF EACH BOX REPRESENTS THE MEDIAN, WHEREAS THE BOTTOM AND TOP OF EACH BOX INDICATES THE FIRST AND THIRD QUARTILES, RESPECTIVELY. THE WHISKERS EXTENDING FROM EACH BOX INDICATE THE MAXIMUM AND MINIMUM VALUES AND THE BLACK DOTS ARE OUTLIERS. BOXPLOTS MARKED WITH DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (T TEST, $P < 0.05$). 74
FIGURE 2-12. BOXPLOTS OF WING AREA (MM^2) OF MALE FTC BY (A) DIET: SD = STANDARD DIET, SDA = STANDARD DIET + TREMBLING ASPEN FOLIAGE, (B) FEEDING REGIME: F = FULL REGIME, S = STARVED, (C) INFECTION: UNINFECTED & INFECTED, AND (D) ORIGIN: ALBERTA & ONTARIO. THE MIDLINE OF EACH BOX REPRESENTS THE MEDIAN, WHEREAS THE BOTTOM AND TOP OF EACH BOX INDICATES THE FIRST AND THIRD QUARTILES, RESPECTIVELY. THE WHISKERS EXTENDING FROM EACH BOX INDICATE THE MAXIMUM AND MINIMUM VALUES AND THE BLACK DOTS ARE OUTLIERS. BOXPLOTS MARKED WITH DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (T TEST, $P < 0.05$). 75
FIGURE 2-13. ALLOMETRIC RELATIONSHIPS BETWEEN MOTH WEIGHT (MG) AND WING AREA (MM^2) OF FEMALE FTC MOTHS BY (A) DIET = SD, (B) DIET = SDA, (C) INFECTION = N, (D) INFECTION = Y, (E) ORIGIN = ONTARIO, (F) ORIGIN = ALBERTA, AND (G) FEEDING REGIME = F. 76
FIGURE 2-14. ALLOMETRIC RELATIONSHIPS BETWEEN MOTH WEIGHT (MG) AND WING AREA (MM^2) OF MALE FTC MOTHS BY (A) DIET = SD, (B) DIET = SDA, (C) INFECTION = N, (D) INFECTION = Y, (E) ORIGIN = ONTARIO, (F) ORIGIN = ALBERTA, (G) FEEDING REGIME = F, AND (H) FEEDING REGIME = S. 77
FIGURE 2-15. PYRAMID PLOTS OF INFECTION LOAD (LOW = 1 < SPORES < 100, HIGH = 100+ SPORES PER 10 FIELDS OF VIEW AT 400X MAGNIFICATION) IN FTC BY (A) DIET: SD = STANDARD DIET, SDA = STANDARD DIET + 1% LYOPHILIZED TREMBLING ASPEN FOLIAGE, (B) FEEDING REGIME: F = FULL REGIME, S = STARVED, (C) ORIGIN: ORIGIN:

Alberta & Ontario, and (D) Infection Status: Naturally infected and Inoculated at the 3rd larval instar. 78
Figure 3-1. Interaction plot displaying mean wing area (mm^2) ± SE by Origin (AB = Alberta, OD = Ontario – Dryden, OK = Ontario – Kenora) and grouped by Diet (Aspen, Diet) of male FTC moths. Total wing area obtained by adding the areas of the right forewing and the right hindwing. Pariwise comparisons were computed with a Kenward-Roger approximation method for degrees of freedom and a Tukey p-value adjustment. AB-Diet was significantly different from AB-Aspen ($T_{481} = 3.881$, $p = 0.002$) and OD-Aspen was significantly different from OD-Diet ($T_{383} = 3.864$, $p = 0.002$). No difference was found between OK-Aspen and OK-Diet ($T_{484} = 0.812$, $p = 0.965$). To obtain moth wing area, the right forewing and the right hindwing measurements were added together. 2014 data. 116
Figure 3-2. Interaction plot displaying mean wing area (mm^2) ± SE by Origin (AB = Alberta, OD = Ontario – Dryden, OK = Ontario – Kenora) and grouped by Diet (Aspen, Diet) of female FTC moths. Total wing area obtained by adding the areas of the right forewing and the right hindwing. Pariwise comparisons were computed with a Kenward-Roger approximation method for degrees of freedom and a Tukey p-value adjustment. AB-Diet was significantly different from AB-Aspen ($T_{363} = 3.418$, $p = 0.009$) and OD-Aspen was significantly different from OD-Diet ($T_{304} = 5.035$, $p < 0.001$). No difference was found between OK-Aspen and OK-Diet ($T_{357} = 0.516$, $p = 0.996$). To obtain moth wing area, the right forewing and the right hindwing measurements were added together. 2014 data. 117
Figure 3-3. Boxplots of wing area (mm^2) of (A) male and (B) female FTC moths by Infection (N = uninfected, Y = Infected). Total wing area obtained by adding the areas of the right forewing and the right hindwing. The midline of each box represents the median, whereas the bottom and top of each box indicates the first and third quartiles, respectively. The whiskers extending from each box indicate the maximum and minimum values and the black dots are outliers. Boxplots marked with different letters are significantly different (F statistic, $P < 0.05$). 2014 data. 118
Figure 3-4. Boxplots of wing area (mm^2) of (A) male and (B) female FTC moths by Infection Load: None = 0 spores, Low = 1 < spores < 100, High = 100+ spores per 10 fields of view at 400x magnification. Total wing area obtained by adding the areas of the right forewing and the right hindwing. The midline of each box represents the median, whereas the bottom and top of each box indicates the first and third quartiles, respectively. The whiskers extending from each box indicate the maximum and minimum values and the black dots are outliers. Boxplots marked with different letters are significantly different (t test, $P < 0.05$). 2014 data. 119
Figure 3-5. Pyramid plots of infection load (Low = 1 < spores < 100, High = 100+ spores per 10 fields of view at 400x magnification) in FTC by (A) Sex: F = Females, M = Males, (B) Origin: AB = Alberta, OD = Ontario – Dryden, OK = Ontario – Kenora, and (C) Diet: Aspen and Artificial Diet. 120
Figure 3-6. Boxplots of wing area (mm^2) of male (A) and female (B) FTC moths by Infection: Uninfected & Infected. ½ of the total wing area was used. The midline of each box represents the median, whereas the bottom and top of each box indicates the first and third quartiles, respectively. The whiskers extending from each box indicate the maximum and minimum values and the black dots are outliers. Boxplots marked with different letters are significantly different (F statistic, $P < 0.05$). 2018 data. 121
Figure 3-7. Boxplots of wing area (mm^2) of male (A) and female (B) FTC moths by diet: FA = Fresh Aspen, FM = Fresh Maple, SD = Standard Diet. ½ of the total wing area was used. The midline of each box represents the median, whereas the bottom and top of each box indicates the first and third quartiles, respectively. The whiskers extending from each box indicate the maximum and minimum values and the

black dots are outliers. Boxplots marked with different letters are significantly different (t test, P < 0.05). 2018 data. 122
Figure 3-8. Boxplots of wing area (mm^2) of male (A) and female (B) FTC moths by infection load: None: 0 spores count, Low: 1-100 spores count, High: 101+ spores count per 10 fields of view at 400x magnification. ½ of the total wing area was used. The midline of each box represents the median, whereas the bottom and top of each box indicates the first and third quartiles, respectively. The whiskers extending from each box indicate the maximum and minimum values and the black dots are outliers. Boxplots marked with different letters are significantly different (t test, P < 0.05). 2018 data. 123
Figure 3-9. Pyramid plots of malformed wings in FTC by (A) Sex: F = Females, M = Males, (B) Diet: FA = Fresh Aspen, FM = Fresh Maple, and SD = Standard Diet, (C) Infection: N = uninfected, Y = infected, and (D) Infection Load: None: 0 spores count, Low: 1-100 spores count, High: 101+ spores count per 10 fields of view at 400x magnification. 2018 data. 124
Figure 3-10. Pyramid plots of infection load (Low = 1 < spores < 100, High = 100+ spores per 10 fields of view at 400x magnification) in FTC by (A) Sex: F = Females, M = Males, and (B) Diet: FA = Fresh Aspen, FM = Fresh Maple, SD = Standard Diet. 2018 data. 125

List of Abbreviations

AB = Alberta

Btk = *Bacillus thuringiensis sp. kurstaki* Berliner (Bacillales: Bacillaceae)

F = Full feeding regime

FA = Fresh trembling aspen foliage

FM = Fresh sugar maple foliage

FTC = Forest tent caterpillar

GLMM = Generalized Linear Mixed-Effects Model

High = Infected (100+ spores per 10 fields of view at 400x magnification)

LMM = Linear Mixed Effect Model

Low = Infected (1 to 100 spores per 10 fields of view at 400x magnification)

N = Uninfected

None = Uninfected (0 spore count per 10 fields of view at 400x magnification)

NPV = Nucleopolyhedrovirus

OD = Ontario – Dryden

OK = Ontario – Kenora

S = Partially starved

SD = Standard diet (Addy, 1969)

SDA = Standard diet amended with 1% lyophilized trembling aspen foliage

Y = Infected

YY = Infected at birth

Chapter 1 – General Introduction

Outbreaking forest lepidoptera

One of the major areas of study in ecological research concentrates on understanding what influences recurring changes in size and structure of populations over time. Ecologists have been puzzled with population fluctuations since the early 1900s and have developed several mathematical models on the matter (Nicholson & Bailey, 1935). Numerous hypotheses have been postulated to describe drivers of population fluctuations; however, the complexity of these systems includes a combination of biotic, abiotic, and multitrophic factors that likely dictate population dynamics. Some of the most extreme examples of cyclical population fluctuations are offered by forest Lepidoptera. Myers & Cory (2013) identified the major requirements for population cycling; high reproductive potential that allows rapid population growth, density-related mortality factors, and delayed mechanisms that negatively affect subsequent population growth. Furthermore, population cycles are often characterized by specific patterns, which include a relatively long phase of population increase, a period of peak density, and a subsequent population decline (Myers, 1988).

The interest in the cyclical dynamic patterns of forest Lepidoptera populations is not only driven by scientific curiosity, but also by quantifiable ecological and economic damage. For example, the forest sector provides a major contribution to Canada's economy. In 2019, the forest sector generated CAD $23.7 billion to Canada's nominal gross domestic product (GDP), with a CAD $33 billion annual export market value, and over 200 000 jobs (Natural Resources Canada, 2020a). Moreover, Canada's 347 million hectares of forest represent 9% of the world's forests (Natural Resources Canada, 2020b), which create a wide array of ecosystems, promote carbon sequestration, produce oxygen, and provide a variety of other ecosystem functions. On average, between 2000 and 2018, insects accounted for over 16.4 million hectares (ha) of forest defoliation in Canada alone (Varrella, 2021). In 2018, almost 6

million ha were defoliated by the spruce budworm *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae), over 1.5 million by the forest tent caterpillar, *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae), and over 1.1 million by the jack pine budworm, *Choristoneura pinus* Freeman (Lepidoptera: Tortricidae) (Natural Resources Canada, 2020c). It is important to remember, however, that these numbers undergo continuous fluctuations depending on the stage of the population cycle of the insect. For example, in 2013, the area defoliated by the spruce budworm was only half (2.8 million ha) of that reported in 2018. On the contrary, in 2013 the forest tent caterpillar caused almost 7.5 million ha of forest damage, five times as much as the damage recorded in 2018. For these reasons, special interest has been given to understanding and, potentially, managing forest Lepidoptera population cycles.

Long-term studies of populations indicate that the increase phase of lepidopteran population cycles is subject to variation in rate, timing, and density among populations within a specific geographical area (Myers, 1988). While the decline phase shows more synchrony and appears to affect regional populations simultaneously. This peculiar behaviour has been observed in several outbreaking Lepidoptera, such as the spruce budworm (Royama, 1984), the larch budmoth *Zeiraphera diniana* Guenée (Lepidoptera: Tortricidae) (Baltensweiler et al., 1977), the western tent caterpillar *Malacosoma californicum* Packard (Lepidoptera: Lasiocampidae) (Wellington, 1960), the forest tent caterpillar (Witter et al., 1975), and the Douglas-fir tussock moth *Orgyia pseudotsugata* McDunnough (Lepidoptera: Erebidae) (Mason, 1974), as reported by Myers (1988). The synchronous behaviour of population decline suggests the presence of one or more shared factor(s) that influence all populations simultaneously. Several hypotheses have been postulated to explain this phenomenon, including density driven self-regulating mechanisms, top-down effects caused by predators, pathogens, and parasitoids, bottom-up effects relative to plant quality and induced chemical defenses, and climatic fluctuations (Myers, 2013). Predation exclusion experiments on outbreaking Lepidoptera suggest that predation

does not play a major role in reducing high density populations. Instead, predators are more important in the control of low-density populations (Torgersen, 1985). High population density is linked to an increase in parasitoids, another top-down effect with the potential to regulate population dynamics. For example, parasitoid-exclusion experiments of the autumnal moth, *Epirrita autumnata* Borkhausen (Lepidoptera: Geometridae), allowed high density populations to occur for longer periods of time compared to natural populations (Klemola et al., 2010). Entomopathogens cause slow-acting chronic effects (Becnel & Andreadis, 1999; Rothman & Myers, 1996), that might function as a delayed negative mechanism to prevent or delay subsequent population growth. But results of studies on the effect of entomopathogens on population decline in forest Lepidoptera are equivocal and, in some cases, there is no relationship between pathogens and population decline (Baltensweiler et al., 1977; Mason & Torgersen, 1987).

There is also evidence of bottom-up effects that influence the qualitative and quantitative nutritional uptake of larvae and contribute to the regulation of population cycles in outbreaking forest Lepidoptera. Plants are known to respond to herbivory by producing secondary compounds (Rhoades, 1985), which can cause detrimental effects on phytophagous insects (Boggs, 1992; Roff, 2009; Scriber & Slansky, 1981), and may even alter resource allocation to favour dispersal over reproduction (Soule et al., 2020). Even though there is evidence to support the effect of bottom-up regulation of population cycling, just as much evidence contradicts it (Nykänen & Koricheva, 2004). Primary and secondary metabolites in plants fluctuate depending on a variety of biotic and abiotic factors (Hwang & Lindroth, 1997; Lindroth et al., 1993; Lindroth & Hwang, 1996; Osier et al., 2000; Salminen et al., 2004), suggesting climatic conditions may affect Lepidoptera population dynamics both directly and indirectly. It is clear that a single generalized hypothesis is not sufficient to explain the complexity of the population dynamics of outbreaking forest Lepidoptera. As suggested by Myers & Cory (2013), future studies should explore multitrophic interactions as factors that drive population cycling. Understanding population

dynamics may help us predict and regulate outbreaks, ultimately contributing to forest health and the economy that depends on the forest industry.

***Malacosoma disstria* Hübner**

The forest tent caterpillar (FTC), *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae), is one of the most important native defoliators of deciduous hardwood trees in North America (Fitzgerald, 1995). The FTC is the most widespread tent caterpillar in its geographical range, and it feeds on a variety of host species depending on its location (Baker, 1972). It differs from other tent caterpillars (genus *Malacosoma*) in that it does not produce silken tents, instead, FTC larvae create silken mats on which they aggregate to molt and rest. The FTC is univoltine; first instar larvae overwinter within eggs in masses and emerge synchronously with host tree bud burst in early spring. Young larvae move together on silken paths following pheromone trails for foraging (Fitzgerald & Costa, 1986). This gregarious behaviour takes place from egg hatch until fifth instar, at which point larvae switch to independent foraging behaviours. The behavioural switch during juvenile development is driven by the costs of group living, including costs of predation, intraspecific resource competition, and silk production (Despland & Le Huu, 2007). Forest tent caterpillar larvae reach full size (up to 64 mm) in 5 to 6 weeks from egg hatch (Bieman & Witter, 1983). The larvae spin silken cocoons in sheltered places for pupation. After about 10 days, adult males are the first to eclose, and search for mates. Like most moth species, FTC uses a female-produced sex pheromone in mate location (Struble, 1970). Females conduct an oviposition flight to locate a suitable place to lay eggs within a few hours of copulation. The adults do not feed, and die within a few days after eclosion (Fitzgerald, 1995). Eggs are laid in masses and are protected by a spumaline coating. Each egg mass may contain 50-300 eggs, in which embryos develop for ~3 weeks before overwintering as first instar larvae.

Forest tent caterpillars, like many other forest Lepidoptera, undergo cyclical fluctuations in population density, during which, peaks of larval abundance can cause stand-level defoliation

(Fitzgerald, 1995). Outbreaks are cyclical and occur in 6 to 16-year intervals depending on the geographical range (Duncan & Hodson, 1958; Cooke et al., 2009). Outbreak patterns present increasing interval length and decreasing synchrony from East to West across North America. For example, in Québec, outbreaks occur with a high degree of periodicity at 10-year intervals (Cooke & Lorenzetti, 2006). In Ontario, outbreaks recur on 13-year cycles (Daniel & Myers, 1995), whereas in Manitoba they average 14-to-17-year cycles (Sutton & Tardif, 2007). In western Canada, outbreaks are asynchronous and often lack periodicity (Cooke, 2001; Cooke & Roland, 2018; Hogg et al., 2002). Severe defoliation usually persists for 2 to 4 years, and can limit tree growth (Hogg et al., 2002), and more rarely, even lead to tree mortality (Brandt et al., 2003). In 1991, FTC defoliated 19 million ha of forest throughout Canada, but less than 1 million ha two years later (Natural Resources Canada, 2020). Several factors contribute to the initiation of FTC population outbreaks, including favourable temperatures during insect development, phenological synchrony with host plants, and low predation and parasitism by natural enemies (Cooke & Roland, 2000; Cooke & Roland, 2018; Daniel & Myers, 1995; Ives, 1973; Myers & Cory, 2013; Witter et al., 1972; Witter et al., 1975). Heterogeneity of forests and increased temperatures promote longer and more severe outbreaks (Cooke & Roland, 2000; Roland et al., 1998). Population outbreaks of FTC result in changes in resource allocation between flight and reproduction (Evenden et al., 2015; Jones & Evenden, 2008) and pheromone-mediated mating behaviours (Evenden et al., 2015) in adult moths. Male FTC moths initiate mating behaviour earlier in the day in high density populations, which permits females to choose mates (Bieman & Witter, 1983). Recent studies (Dukes et al., 2009; Uelmen et al., 2016; Cooke & Roland, 2018) suggest FTC has the potential to become more persistent as a result of climate change. Rising temperatures are associated with increased synchronization in budburst date among tree species (Jeong et al., 2013), which may lead to a more synchronized larval emergence (Bellemin-Noël et al., 2020) and longer and more severe outbreaks (Cooke & Roland, 2000; Roland et al., 1998).

Host Use

The forest tent caterpillar is a polyphagous insect that is able to feed on the foliage of a variety of hardwood trees in the genera *Populus* L., *Acer* L., *Quercus* L., *Liquidambar* L., and *Nyssa* G. (Batzer & Morris, 1978; Parry & Goyer, 2004). Geographically distinct populations, however, display a degree of adaptation on specific host species (Parry & Goyer, 2004), to which they are phenologically synchronized (Gray & Ostaff, 2012). Furthermore, oviposition and early larval feeding behaviours display a more restricted and selective host affiliation than older larvae (Parry & Goyer, 2004). The main host of FTC in Canada and the western USA is trembling aspen, *Populus tremuloides* Michx (Malapighiales: Salicaceae), although larvae feed on a variety of secondary hosts, especially at high densities (Furniss & Carolin, 1977). In the eastern North America, FTC feeds on sugar maple, *Acer saccharum* Marshall (Sapindales: Sapindaceae), northern red oak, *Quercus rubra* Linnaeus (Fagales: Fagaceae), as well as trembling aspen (Parry & Goyer, 2004). In the southern USA, water tupelo, *Nyssa aquatica* Linnaeus (Cornales: Nyssaceae), black tupelo, *Nyssa sylvatica* Marshall (Cornales: Nyssaceae), and American sweetgum, *Liquidambar styraciflua* Linnaeus (Saxifragales: Altingiaceae), are most affected by FTC defoliation (Harper & Hyland, 1981; Wagner, 2010). Even though FTC presents some degree of host affiliation within its geographical range, host-induced factors, such as primary (Colasurdo et al., 2009) and secondary (Jocelyn & Lindroth, 2000) metabolites, and budbreak phenology (Parry et al., 1998), impact FTC fitness. Tree host exposure to atmospheric gasses can also alter FTC-host interactions. For example, exposure to enhanced O_3 levels during plant development increases FTC larval preference to paper birch, *Betula papyrifera* Marshall (Fagales: Betulaceae), compared to its primary host, trembling aspen (Agrell et al., 2005). On the contrary, Noseworthy & Despland (2006) reported feeding choice is largely dictated by pheromone trails, even when higher and lower quality diets are compared.

In Canada and the northern USA, the primary host of FTC is trembling aspen. Trembling aspen naturally extends from Canada to central Mexico, and it is one of the most ecologically and economically

important deciduous hardwood trees in North America (Peterson & Peterson, 1992). Its commercial application varies from pulp and paper production to medicine, construction, packing, and animal bedding, among others. Forest tent caterpillar is the most important defoliator of trembling aspen (Peterson & Peterson, 1992), and FTC defoliation has been linked to increased aspen mortality during outbreaks (Man et al., 2008). Damage by FTC can have detrimental economic impacts, including delayed tree growth that impacts harvest rotations (Man & Rice, 2010). FTC is also an important disturbance agent in trembling aspen-dominated forests. Due to its abundance and distribution, trembling aspen provides important ecological services to Canada's boreal forest. Aspen is a pioneer species that grows quickly and promotes recovery of ecosystems damaged by natural and/or human disturbances, such as wildfires and deforestation (Gylander et al., 2012). Trembling aspen is considered an integral part of boreal forest management (Dancik, 1990).

Trembling aspen produces a wide array of secondary metabolites associated with chemical defense against herbivory (Lindroth et al., 1987b; Palo, 1984). Among them, phenolic glycosides like salicin, salicortin, tremuloidin, and tremulacin impact FTC performance (Hwang & Lindroth, 1997). Phenolic glycosides can range between 1 – 8% of leaf dry weight, depending on genetic and environmental factors (Lindroth & Hwang, 1996; Osier et al., 2000). Trembling aspen also contains a variable amount of condensed tannins, which can make up 3 to nearly 30% of leaf dry weight (Bryant et al., 1987; Lindroth & Hwang, 1996; Osier et al., 2000; Schultz et al., 1982). Variation in trembling aspen secondary chemistry can be affected by tree age (Erwin et al., 2001) and genetics (Lindroth & Hwang, 1996; Osier & Lindroth, 2001), as well as seasonal environmental conditions and phenology (Bryant et al., 1987; Lindroth et al., 1987a). Trembling aspen chemical defenses can also be induced by herbivory (Peters & Constabel, 2002). Secondary chemicals in trembling aspen foliage have detrimental effects on FTC survival, growth rate, food consumption, approximate digestibility, and nutrient processing efficiency (Hemming & Lindroth, 1995; Hwang & Lindroth, 1997; Jocelyn & Lindroth, 2000; Lindroth &

Bloomer, 1991). For example, fourth instar FTC larvae reared on trembling aspen leaves supplemented with 4% phenolic glycosides display slower development time and reduced growth rates (Jocelyn & Lindroth, 2000).

Sugar maple is also an important forest tree species in Canada. Canada's production and export of sugar maple products accounts for roughly 75% of the global market, which provided a gross value of over CAD$ 558 million in 2020 alone (Statistics Canada, 2021). Forest tent caterpillar defoliation has been linked to reduced quality and quantity of sugar maple sap (Winch & Morrow, 1989), representing an economic threat to the maple syrup industry. Moreover, FTC outbreaks are associated with sugar maple decline (Wood et al., 2009). The economic value of sugar maple is not limited to syrup production, as this species is also widely used in hardwood lumber production (Niese & Strong, 1992).

Sugar maple foliage is also characterized by a wide array of secondary metabolites. Condensed tannins constitute ~ 20% of foliage dry weight (Schultz et al., 1982) but quantities vary with tree genetics, geographical range, and season (Baldwin et al., 1987; Schultz et al., 1982). Sugar maple foliage also contains hydrolysable tannins such as ellagitannins, gallic acid, and gallotannins, which are not present in trembling aspen (Baldwin et al., 1987; Lindroth et al., 1987; Lindroth et al., 1993). Chemical differences between sugar maple and trembling aspen impact FTC host choice and fitness. Forest tent caterpillar larvae display higher pupation rates, larval and pupal weight, and faster development time on trembling aspen compared to sugar maple (Nicol et al., 1997). Furthermore, FTC reared on trembling aspen foliage have increased fecundity and lay bigger eggs than those laid by FTC reared on sugar maple (Trudeau et al., 2010). Forest tent caterpillar larvae prefer diet treated with trembling aspen extracts compared to sugar maple extracts (Panzuto et al., 2001), which is likely driven by variation in tree chemistry. Early spring trembling aspen foliage contains ~ 8.5% soluble sugars, which is more than double the concentration found in sugar maple, about 3.5% (Lorenzetti, 1993). Moreover, the

concentration of condensed tannins in trembling aspen is significantly lower than the concentration found in sugar maple foliage (Bryant et al., 1987; Schultz et al., 1982).

Natural Control

A wide array of natural abiotic and biotic factors has been linked to population declines in FTC. Cold temperatures are associated with high mortality at all developmental stages. Extremely cold winters can lead to egg mortality (Cooke & Roland, 2003). Freezing temperatures during and following hatch can suppress young larvae, and extreme fluctuations in temperatures during the adult stage can interfere with mating success and reduce fertility (Fitzgerald, 1995; Levesque et al., 2002). At high population densities, FTC can starve due to intraspecific competition for resources (Fitzgerald, 1995; Sutton & Tardif, 2007).

Several predators and parasites are known to affect FTC populations (Witter & Kuhlman, 1972). Predators are generally recognized to play an important role in maintaining low population densities but cause limited mortality during the peak phase of population cycling (Glasgow, 2006). The opposite trend is observed in parasitoids, which generally provide a delayed density-related mortality factor through tracking FTC population size (Roland, 2005). A wide array of predators feed on FTC, including insects, birds, mammals, and amphibians. Bird predation is a major regulator of FTC at endemic populations (Parry et al., 1997) and it is hypothesized that birds are the primary predators of late instar larvae (Nixon & Roland, 2012). Parry et al., (1997) attributed the increased predation on late instar larvae to the nesting of several species of songbirds, which prey on FTC to feed their nestlings. At least 60 species of birds have been reported to feed on FTC (Witter & Kuhlman, 1972). On the contrary, early instar predation is predominantly caused by arthropods (Nixon & Roland, 2012).

There are at least 14 hymenopteran egg parasitoids, along with 61 hymenopteran and 52 dipteran larval and pupal parasitoids of tent caterpillars in the genus *Malacosoma* (Witter & Kuhlman,

1972). *Telenomus clisiocampae* Riley (Hymenoptera: Scelionidae), *Ooencyrtus clisiocampae* Ashmead (Hymenoptera: Encyrtidae), and *Baryscapus malacosomae* Girault (Hymenoptera: Eulophidae) are common FTC egg parasitoids (Williams & Langor, 2011; Witter & Kuhlman, 1972). The parasitic fly *Sarcophaga aldrichi* Parker (Diptera: Sarcophagidae) may act as a delayed density-dependent mortality factor (Sippell, 1962) and is also associated with transmission of nucleopolyhedroviruses (NPV) (Stairs, 1966) in FTC. *Leschenaultia exul* Townsend (Diptera: Tachinidae) is one of the most common larval parasitoids in FTC at endemic populations (Parry, 2005) and can exert a high density-related influence by responding numerically after the second year of high host population density (Parry, 2005; Parry et al., 1997). Large numbers of *Aleiodes malacosomatos* Mason (Hymenoptera: Braconidae) at endemic FTC populations may control population dynamics at low density (Parry et al., 1997). *Patelloa pachypyga* Aldrich & Webber (Diptera: Tachinidae) may also contribute to controlling FTC at low densities (Parry et al., 1997) but it can be outcompeted by faster developing parasitoids (Parry, 1995). The ability of parasitoids to exert influence on FTC is closely linked to parasitism strategy (Roland & Taylor, 1997) and timing of attack (Parry, 1995). Moreover, forest structure plays a major role in influencing parasitism success (Roland & Taylor, 1997).

Some of the most fascinating natural enemies of FTC, however, are pathogens, such as viruses, bacteria, fungi, and microsporidia (see below). Nucleopolyhedrovirus (NPV) is an important mortality factor of FTC (Keddie & Erlandson, 1995), especially at high population densities (Myers, 1993). NPV is recognized as one of the major driving forces in regulating FTC population dynamics, and it has often been associated to population collapse during outbreaks (Myers, 1993). NPV enters the insect in crystal form via ingestion and is dissolved by the alkaline pH of the gut (Clark, 1958). The virus then penetrates cell membranes and affects cell nuclei throughout the insect. FTC infected with NPV usually die within 10 to 14 days from exposure (Roland & Kaupp, 1995). Transmission rate is lower at forest edges and the top of canopy trees (Roland & Kaupp, 1995), which is likely the result of higher UV radiation that

inactivate the virus outside the host (Broome et al., 1974). For this reason, Roland & Kaupp (1995) hypothesized female FTC lay eggs on the higher branches of trees as a behavioural adaptation to avoid NPV infection. Six different isolates of NPV naturally occur in FTC populations (Ebling & Kaupp, 1995), which can be transmitted vertically (from mother to offspring) and horizontally directly through foraging (Stairs, 1966). Vertical transmission is thought to be facilitated by the parasitoid fly *S. aldrichi* (Stairs, 1966).

Other naturally occurring pathogens are recognized to cause mortality in FTC, such as the entomopathogenic fungi *Beauveria bassiana* Vuillemin (Hypocreales: Cordycipitaceae) (Stark & Harper, 1982), and *Furia gastropachae* Raciborski (Zygomycetes: Entomophthorales) (Filotas et al., 2003). Bacteria in the genera *Bacillus, Clostridium*, and *Serratia* are also naturally occurring in FTC, but they offer limited control under natural conditions, as reported by Witter & Kulman (1972).

Microsporidia

Microsporidia are unicellular eukaryotic parasites first recorded in the 1800s (Moniez, 1887; Pasteur, 1870). They were originally thought to be protists (Cavalier-Smith, 1993; Corliss & Levine, 1963) but later reclassified as fungi (Fischer & Palmer, 2005; Hibbett et al., 2007) or belonging to a sister group of fungi (Lee et al., 2008). The phylum Microsporidia includes 1300 to 1500 known species (Vávra & Lukeš, 2013) that are parasitic on most animal groups. They most commonly infect arthropods and fish, but they also cause microsporidiosis in human patients with acquired immunodeficiency syndrome (AIDS) (Weber et al., 2000). Microsporidia are among the smallest eukaryotes; they lack mitochondria, peroxisomes, and centrioles, and possess extremely small eukaryotic genomes (Corradi et al., 2010). Outside the host, a wall composed of an exospore and an endospore protects spores from environmental extremes, making them virtually ubiquitous (Bigliardi et al., 1996). Spore size and shape vary with species, but microsporidia are generally characterized by an oval or pyriform shape, ranging between 1 and 40µm (Weiss & Becnel, 2014). Spores can enter the host via horizontal (direct contact) or

vertical (from parental organism) transmission. Upon infection, spores build osmotic pressure, which bursts the cell wall allowing the ejection of the polar filament (or polar tube) (Keeling & Fast, 2002). The polar filament is propelled outside the spore with sufficient force to pierce the cell membrane of the host. There, it functions as a "needle" for the transmission of sporoplasm into the cytoplasm of the host cell. The sporoplasm replicates through merogony to create a multinucleate plasmodium. These new nuclei will undergo sporogony and produce new spores (Vávra & Larsson, 1999). Depending on the species, the cycle can vary, but it generally repeats until the cell is completely colonized (Weiss & Becnel, 2014).

In insects, microsporidia have been identified as a source of disease since the mid 1800s. Pasteur (1870) associated the silkworm disease (or Pébrine) to infection by a protist, later reclassified as the microsporidium *Nosema bombycis* (Dissociodihaplophasida: Nosematidae) (Balbiani, 1882). Microsporidian infection in insects is highly variable but can be generalized into two categories: 1) fast acting (or acute), which generally results in death; or 2) slow acting (or chronic), which causes slow, detrimental sublethal effects (Weiss & Becnel, 2014). Most of the damage to the host occurs internally, but external symptoms are sometimes visible, such as reduction in size and weight (Linde et al., 1998; Thomson, 1958; Wilson, 1983; Wilson, 1977; Wilson, 1984), changes in colouration (HabteWold et al., 1995), malformations of the body (Tounou, 2007), and alterations in insect behaviour (Graystock et al., 2013). Internally, microsporidia develop within the cytoplasm of the host insect's cells, most commonly in the fat body and midgut epithelium, but other tissues may also be infected. As high concentrations of microsporidia can cause insect mortality (Thomson, 1959), microsporidia have been examined as potential biological control agents. Several studies have focused on the effects of microsporidia on a variety of pest species, in both forest and agriculture landscapes (Bomar et al., 1993; Briano et al., 2002; Lewis et al., 2006; Solter et al., 2002; Thomson, 1959; Weiser & Novotný, 1987), and some field applications have been attempted. For example, concentrated solutions of *Paranosema (Nosema)*

locustae (Dissociodihaplophasida: Nosematidae) sprayed in Tianjin, China resulted in a 97.5% reduction of the Oriental migratory locust, *Locusta migratoria manilensis* Meyen (Orthoptera: Acrididae) (Fu et al., 2010). Field applications of *Nosema lymantriae* (Dissociodihaplophasida: Nosematidae) greatly reduced the number of larvae, pupae, and adults of the gypsy moth, *Lymantria dispar* Linnaeus (Lepidoptera: Erebidae) (Weiser & Novotný, 1987). Research on microsporidia application as a biological control measure is still limited, likely due to their slow-acting sublethal effects and limits to mass production (Canning, 1982).

Microsporidia are now recognized to be present in most forest defoliators, and to contribute to natural control of populations in a wide array of forest Lepidoptera (Maddox et al., 1998). Therefore, microsporidia may be a contributing factor leading to the cyclical population dynamics of forest Lepidoptera. For example, *Nosema fumiferanae* (Dissociodihaplophasida: Nosematidae) infection in the spruce budworm delays spring larval emergence, which reduces its ability to disperse (van Frankenhuyzen et al., 2007). An increase of *Nosema* sp. (Dissociodihaplophasida: Nosematidae) in jack pine budworm is correlated with high larval densities, suggesting it may play a role in dictating cyclical outbreaks patterns (van Frankenhuyzen et al., 2011). For these reasons, microsporidian infections in outbreaking forest Lepidoptera should not be overlooked; they may help us better understand the driving forces behind cyclical occurrence of outbreaks.

Forest tent caterpillars are commonly infected with spores of *Nosema disstriae* (Thomson, 1959), and have been found to be susceptible to *Pleistophora schubergi* (Wilson, 1977), *Vairimorpha necatrix* (Wilson, 1984), and *Thelohania pristiphorae* (Smirnoff, 1968). Microsporidian infections in FTC cause reduced body size and activity, decreased fecundity and longevity, and increased mortality (Thomson, 1958; Wilson, 1977; Wilson, 1984). The rate and intensity of microsporidian infection in FTC varies with the geographic range of the insect (Jones & Evenden, 2008), as well as density (Fitzgerald,

1995). It is likely that microsporidia interact with other ecological factors to influence FTC population cycling.

Diet-Disease Interactions

Recent studies have explored diet-disease interactions and their potential implications in the control of outbreaking pests. Evidence suggests differences in plant quality and plant chemistry can alter the susceptibility of Lepidoptera to entomopathogens, as well as modify the rate of replication of pathogens within the insect (Cory & Hoover, 2006). Results are, however, equivocal and the mechanisms behind these tritrophic interactions remain largely unknown.

The pathogenicity and replication of nucleopolyhedroviruses (NPV) are significantly affected by insect-host affiliation and it appears to be regulated by concentrations of secondary chemicals. For example, condensed (Lindroth et al., 1999; Hunter & Schultz, 1993) and hydrolysable (Keating et al., 1988) tannins are linked to reduced susceptibility and mortality by NPV infection in the gypsy moth (Lepidoptera: Erebidae). On the contrary, salicin from trembling aspen significantly increases larval mortality in the same system (Cook et al., 2003). This suggests phenolic glycosides interact with NPV in a different fashion compared to hydrolysable and condensed tannins. Similar findings have been reported in other plant – entomopathogen systems concerning *Bacillus thuringiensis sp. kurstaki* Berliner (Bacillales: Bacillaceae) (*Btk*). Purified tannins from genus *Quercus* L. reduced pathogenicity of *Btk* against the gypsy moth (Appel & Schultz, 1994), however, phenolic glycosides from aspen trees were linked to increased pathogenicity in the same insect (Arteel & Lindroth, 1992; Hwang et al., 1995). Moreover, FTC mortality caused by *Btk* is greater when larvae are fed sugar maple compared to trembling aspen (Kouassi et al., 2001). Sugar maple is characterized by higher phenolic compounds, which would suggest enhanced effects (Arteel & Lindroth, 1992; Hwang et al., 1995), however, sugar maple also presents higher hydrolysable and condensed tannins, which play a role in improving insect resistance to *Btk* (Appel & Schultz, 1994). It is therefore possible that plant quality and primary nutrients

also play a role in shaping the direction of this interaction (Foster et al., 1992), suggesting the presence of a cost of resistance (Janmaat & Myers, 2005).

To our knowledge, Smirnoff (1967) was the first to assess the effects of different plant extracts and juices on the susceptibility to microsporidian infection (*Nosema* sp.) in a Lepidoptera, the ugly-nest caterpillar, *Archips cerasivorana* Fitch (Lepidoptera: Tortricidae). Smirnoff concluded that foliage treated with onion extract greatly inhibited the spread of infection in larvae and pupae of the ugly-nest caterpillar. Moreover, similar, yet weaker, results were observed in caterpillars fed foliage supplemented with mustard, paper birch, and balsam poplar extracts (Smirnoff, 1967). Onion extract, however, did not alter the cabbage looper sensitivity to microsporidian infection (*Vairimorpha* sp.) (Carloye et al., 1998). On the contrary, the same study demonstrated that the supplementation of xanthotoxin to the larval diet significantly delayed mortality by microsporidian infection (Carloye et al., 1998). A recent experiment by van Frankenhuyzen & Liu (2016) showed no changes in the susceptibility of the spruce budworm to *Nosema fumiferanae* infection when provided previous year foliage, a suboptimal source of nutrition.

Thesis Objective

My studies assess the influence of larval diet and microsporidian infection on life history traits of FTC. In Chapter 2, I test the effects of qualitative and quantitative manipulations of larval diet and microsporidian infection on FTC life-history traits, such as development time, pupation success, silk production, adult body size, and resource allocation. In Chapter 3, I test the effects of microsporidian infection and host affiliation on adult moth body size, as measured by wing area. My studies are the first to assess the effects of the tritrophic interaction between larval diet quality and quantity and microsporidian infection in FTC. Investigating the effects of this multi-trophic interaction will improve our understanding of population dynamics of this important ecological disturbance factor of deciduous

hardwood trees in North America.

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Chapter 2 – Effects of larval diet quality and quantity and microsporidian infection on life history traits of the forest tent caterpillar, *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae).

Abstract

Population cycles in the forest tent caterpillar (FTC), *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae), are influenced by a wide array of top-down and bottom-up effects. The interactions among plant phytochemicals, density-induced starvation, and entomopathogenic infections, as drivers of population cycling have received limited research attention. In this study, we are the first to explore the effects of the tritrophic interaction between larval diet quality and quantity and microsporidian infection in FTC. Larvae that originated from populations in Ontario and Alberta were inoculated with known quantities of the microsporidian parasite *Nosema disstria* (Microsporida: Nosematidae) at the third larval instar (Y). Larvae were reared in the laboratory on a combination of larval diet and feeding regime treatments; their growth and development were compared to uninfected larvae (N) fed in a similar manner. Diet treatments consisted of a standard artificial diet (SD), and the same artificial diet amended with 1% lyophilized trembling aspen, *Populus tremuloides* Michx (Malpighiales: Salicaceae), foliage (SDA). Feeding regimes varied the quantity of each diet provided to the larvae and included a fully fed regime (F) *versus* a partially starved (S) feeding regime. Development time, life history traits, and resource allocation were measured for FTC reared under the various diet-infection combinations. The results suggest that there is no interaction between microsporidian infection by inoculation at the third instar and larval diet quality and/or quantity to affect FTC life history traits. There are individual effects, however, of microsporidian infection and larval diet that affect adult life history traits and resource allocation. Microsporidian infection significantly reduced the proportion of pupae that were able to produce a cocoon, likely as a result of impaired silk gland function. Development time and resource allocation between dispersal and reproduction were affected by all factors (feeding regime, larval diet, and microsporidian infection). This provides further evidence that these factors work in

concert, but not interactively, to affect FTC life history traits, with implications as drivers of population dynamics.

Introduction

Insect – plant interactions play a major role in shaping population dynamics of phytophagous insects (Boggs, 1992; Roff, 2009; Scriber & Slansky, 1981). This is indisputably linked to morphological and chemical characteristics of plants and overall host quality. Fluctuations in plant host quality are associated with variation in life history traits of many insect herbivores, including body size, development time, fecundity, and overall fitness (eg: Awmack & Leather, 2002; Hall et al., 2008; Joern & Behmer, 1997; Naya et al., 2007; Teder et al., 2014; Van Huis et al., 2008). Furthermore, host plant quality can indirectly influence population dynamics of phytophagous insects through alterations of immune responses to entomopathogens (Biere & Bennett, 2013; Cory & Hoover, 2006; Price et al., 1980). Parasite virulence in insects is directly correlated with restricted nutritional uptake (Boots, 2000; Brown et al., 2000), and to variation in the qualitative chemical composition of the plant host (Cory & Hoover, 2006). For example, leaf tannins purified from red oak *Quercus rubra* Linneaus (Fagales: Fagaceae) and chestnut oak *Quercus prinus* Linneaus (Fagales: Fagaceae) reduce the effectiveness of the entomopathogenic insecticide *Bacillus thuringiensis* var *Kurstaki* (Bacillales: Bacillaceae) in gypsy moth *Lymantria dispar* Linneaus (Lepidoptera: Erebidae) larvae (Appel & Schultz, 1994), leading to less efficient control measures. It is important to explore how these multi-trophic interactions can affect life history traits and subsequent population dynamics of forest Lepidoptera. This may ultimately help us better understand how cyclical outbreaks of forest Lepidoptera occur, and what driving forces dictate their duration.

A good study organism with which to explore this multilayered ecological interaction among entomopathogens, herbivorous insects, and plant hosts is the forest tent caterpillar *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae). The forest tent caterpillar (FTC) is an important ecological disturbance factor of deciduous hardwood forests in North America (Fitzgerald, 1995). This insect is phenologically synchronized with its preferred hosts, so that first instar larvae emerge after overwintering within the egg to feed on fresh foliage at bud burst (Gray & Ostaff, 2012). Foliage age significantly influences larval development, and early instar larvae that feed on older foliage have reduced fitness (Jones & Despland, 2006). The FTC is polyphagous but exhibits regional specialization on host plant species over its wide geographic range (Parry & Goyer, 2004). Host trees are in the genera *Populus* L., *Acer* L., *Quercus* L., *Liquidambar* L., and *Nyssa* G. (Batzer & Morris, 1978; Parry & Goyer, 2004). In western Canada, the preferred host is trembling aspen, *Populus tremuloides* Michx (Malpighiales: Salicaceae), an economically and ecologically important tree species in North America (Peterson & Peterson, 1992). Disturbance caused by FTC defoliation is greatest at the peak of cyclical population outbreaks (Cooke & Lorenzetti, 2006; Cooke et al., 2012), when large numbers of larvae feed gregariously and cause extensive stand-level defoliation (Fitzgerald, 1995). Outbreaks rarely kill host trees (Anderson, 1960), but severe defoliation can affect tree growth and may lead to crown dieback (Cooke & Roland, 2007; Hogg et al., 2002). Furthermore, severe defoliation can promote secondary pests or fungal pathogens to further damage trees (Churchill et al., 1964; Houston, 1992). For these reasons, several studies have tried to understand the cyclical dynamics of FTC.

Forest tent caterpillar is univoltine and overwinters as a first larval instar within the egg. Most feeding (~80%) is done in the last two larval instar stages (Fitzgerald, 1995). Given the adults are non-feeding, we can assume that environmental conditions at the larval stage will directly affect adult life history traits. Parameters such as temperature, host choice, nutrients, population density, and entomopathogens infections have been widely explored as drivers of FTC population dynamics. Low

temperatures are associated with slower development time, as well as reduced consumption and growth rates in FTC (Levesque et al., 2002). Host choice is also associated with changes in adult life history traits. Forest tent caterpillar reared on trembling aspen leaves develop faster, have greater pupal weight, and higher fecundity compared to larvae reared on sugar maple (Nicol et al., 1997; Trudeau et al., 2010). Similarly, changes in protein to sugar ratios in larval diet alter mortality, development time, growth rates, and resource allocation (Colasurdo et al., 2009; Noseworthy & Despland, 2006). For example, FTC reared on trembling aspen leaves supplemented with sucrose display higher survival and slower growth compared to larvae reared on trembling aspen leaves alone (Noseworthy & Despland, 2006). Furthermore, high carbohydrate diets promote somatic tissue growth over ovary development. The opposite trend is visible when FTC are reared on a high protein diet or a balanced protein-sugar diet (Colasurdo et al., 2009).

Alterations to life history traits and resource allocation in FTC are also linked to population density (Evenden et al., 2015a) and entomopathogens such as viruses (Stairs, 1966), microsporidia (Thomson, 1959), fungi (Filotas et al., 2003), and bacteria (Kouassi et al., 2001). A combination of these parameters, including entomopathogens, resource competition, and larval diet, may also interact to influence adult life history traits and FTC fitness. For example, Kouassi et al., (2001) observed that FTC infected with *B. thuringiensis* variety *kurstaki* HD-1 and reared on trembling aspen were less susceptible to the pathogen than larvae reared on sugar maple *Acer saccharum* Marshall (Sapindales: Sapindaceae). The discrepancy was attributed to the higher amounts of sugars and a lower concentration of secondary metabolites (phenolic compounds and tannins) in trembling aspen compared to sugar maple (Lorenzetti, 1993).

One of the most common entomopathogens and disease-causing agents in FTC are microsporidia (Thomson, 1959). Microsporidia are obligate, intracellular parasites with transmissible spores that cause a chronic, sublethal disease (Becnel & Andreadis, 1999). Microsporidia can be

transmitted from one individual to the other (horizontal transmission) or from an infected female to the offspring (vertical transmission) (Hoch & Solter, 2017). Forest tent caterpillars are commonly infected with spores of *Nosema disstriae* (Thomson, 1959), but are also susceptible to infection by *Pleistophora schubergi* (Wilson, 1977), *Vairimorpha necatrix* (Wilson, 1984), and *Thelohania pristiphorae* (Smirnoff, 1968). The rate and intensity of microsporidian infection in FTC varies with geographic range (Jones & Evenden, 2008) and population density (Fitzgerald, 1995). Studies on other lepidopteran species suggest microsporidia pathogenicity may be influenced by plant host phytochemistry (Carloye et al., 1998; Smirnoff, 1967). To our knowledge, however, no study on FTC has looked at the tritrophic interaction among microsporidia, insect, and larval diet.

Our study explored the tritrophic interaction among the microsporidia species (*N. disstria*), a lepidopteran forest defoliator (FTC), and its primary host plant (trembling aspen). Manipulations of larval diet assessed the effects of density-induced food stress and plant phytochemistry on larval development and adult life history traits. Investigating the effects of this multi-trophic interaction will improve our understanding of population dynamics of this important ecological disturbance factor of deciduous trees in North America.

Materials and Methods

Caterpillar rearing

Forest tent caterpillar egg masses were obtained from two geographic regions, in Alberta and Ontario. In Alberta, egg masses were collected from trembling aspen trees in February 2019 in the Lac La Biche forest region, AB, Canada (54°50'50.9"N 112°13'03.8"W), where high population densities occurred in 2017 and 2018 (Appendix A, Appendix B). In Ontario, egg masses were collected in November 2018 by the Canadian Forest Service Great Lakes Forestry Centre (CFS, Sault-Ste-Marie,

Ontario) in the Macdonald, Meredith, and Aberdeen Additional township areas on trembling aspen trees (46°31'17.6"N 84°00'21.1"W). Ontario egg masses were received in Alberta on 23, May 2019. All egg masses were stored in paper bags at 4°C until use.

From 1 June - 7 July, 2019 FTC egg masses were randomly selected for use in experiments. Egg masses were soaked in a 6% NaClO solution for 2 minutes to remove the spumaline coating the eggs, rinsed for 5 minutes under running dH_2O and washed in a 0.06% NaClO solution for 30 seconds. This was done to prevent any possible horizontal infection with entomopathogens located on the surface of the egg masses. Egg masses were then air dried on a paper towel and placed individually in Petri dishes (100 x 15mm) to obtain first instar larvae. Caterpillars were reared initially in family groups under laboratory conditions at ~22°C under a 16L:8D photoperiod. Groups of first instar larvae were monitored daily and provided with a $1cm^3$ of artificial standard diet modified from the Addy (1969) protocol (Appendix C) as needed. A randomly selected subsample of second instar larvae (n=5) from each family group was screened for microsporidian infection (see below) so that each egg mass could be scored as healthy (n = 90) or infected (n = 7). The remaining larvae were randomly selected from each egg mass and assigned to a group of 10 in a 177 ml plastic cup with holes on the sides and lid for air circulation (n=8 groups of 10 per healthy egg mass; n=4 groups of 10 per infected egg mass). Each cup was assigned to one combination of experimental treatments, based on: 1) Diet : standard artificial diet (SD) or standard diet + lyophilized trembling aspen at 1% concentration by mass (SDA); 2) Feeding Regime: full regime (F) or partial starvation (S); and 3) Microsporidian infection: yes (Y) or no (N) (Figure 2-1). Uninfected larvae in the infected treatment (Y) were manually inoculated (see below). Naturally infected egg masses were only assigned to the infected treatment combinations (n=4 groups). Egg mass provenance was also recorded (Alberta/Ontario). Each pupa (cocoon included) was moved into individual cups (100ml) and monitored daily until eclosion. Eclosed adults and any individuals that died as pupae were assessed for microsporidian infection status.

Preparation of artificial diet

The published Addy diet protocol (Addy, 1969) was modified to increase survival of laboratory-reared larvae (CFS Sault Ste Marie, Appendix C). Two diet treatments were prepared weekly: the standard artificial diet (SD) and the standard diet amended with 1% lyophilized trembling aspen foliage (SDA). The standard diet with trembling aspen (SDA) was created using young trembling aspen foliage collected after bud burst from different locations around Edmonton, Alberta, to ensure genetic variation. Freshly collected foliage was washed in a 0.06% NaClO solution for 5 minutes and rinsed under running dH_2O. The leaves were air dried and placed under UV light for 10 minutes for each side. The petioles were removed, and the leaves were placed in a freeze dryer (Labconco Corp., Kansas City, MO, USA). A pestle and a coarse metal sieve were used to grind the leaves into a fine powder, which was stored at 4^{o}C in a sealed container until use. The powder was then added at 1% concentration by weight in the diet by mixing it with the wheat germ component of the standard diet (SD).

Feeding Regimes

Feeding regimes were assigned by modification of the amount of food provided to the larvae in each cup. Experimental larvae in the full regime (F) treatment were fed two 1cm^3 cubes of either the SD or SDA diets three times per week. Partially starved (S) larvae were fed once per week with two 1cm^3 cubes of either the SD or SDA diet and two 1cm^3 cubes of agar two times per week, to maintain similar humidity conditions across treatments.

Infection levels

To obtain a reliable source of larvae for our infected treatment (Y), third-instar larvae from uninfected egg masses were inoculated with a sub-lethal concentration (5 µl of 5x10^5 spores) of *Nosema disstriae* (Microsporida: Nosematidae) spore solution (Wilson, 1977; Wilson, 1984) through direct pipetting of spores onto the diet. The spore solution was isolated from the naturally infected first instar

larvae using a modified version of the triangulation method by Cole (1970). Insects were homogenized in distilled H_2O, filtered through cheesecloth, and the solution was left to rest for 12 hours at 4°C to separate microsporidia spores from the liquid. The supernatant was removed, and the remaining solution was diluted to the desired concentration. A hemocytometer was used at 400x magnification to verify spore concentration. The inoculated diet was fed to larvae that had been starved for 24 hours, to increase feeding and ingestion of spores. The larvae were allowed to feed on the inoculated diet for 48 hours. Uninfected third instar larvae in the uninfected treatment (N) were given 5 µl of dH_2O on the diet treatment to act as a control. Larvae that came from egg masses that were determined to be naturally infected were also reared and treated in the same manner as the uninfected larvae.

Microsporidian infection assessment

Dead pupae, as well as all insects that eclosed as adults, were assessed for microsporidian infection. The entire pupa or the abdomen of adult moths was crushed with a pestle in a microfuge tube containing 250 µl of dH_2O. Another 250 µl of dH_2O was added to the tube, and the sample was vortexed for 5 seconds. Twenty-five µl of the solution was pipetted onto a microscope slide, which was then covered with a cover slip and sealed with petroleum jelly to prevent evaporation. The slide was examined with microscopy at 400x on 10 fields of view for the presence of *N. disstriae* spores. The total number of spores in the examined area was counted to assess "infection load" (0 = None, 1-100 = Low, 100+ = High, per 10 fields of view at 400x magnification). Individuals for which the infection status did not match the original status assigned were removed from the experiment (i.e., uninfected individuals in infected treatment).

Trait measurements

Larvae that developed to pupation or to adulthood were used to assess FTC susceptibility to variation in larval diet, feeding regime, and microsporidian infection. Egg mass provenance

(Alberta/Ontario) was also included as a factor. Cocoon production (Present/Absent) was evaluated based on a visual assessment of the production of silk around each pupa. When present, cocoons were removed from pupae and weighed to the nearest 0.01 mg (Mettler Toledo XPE205 Microbalance, Columbus, OH, USA). Development time was measured as the number of days between first instar hatch and adult eclosion. Development time was further divided into the number of days required to reach the pupal stage, and the number of days spent in the pupal stage.

To obtain wing area measurements, all four wings were removed from moths and glued onto a white piece of paper. The paper was scanned at 118.1 pixels/cm (300 PPI) in JPEG format. ImageJ software was used to process the wings into a binary black and white image to determine wing area in mm^2. Total wing area was then calculated by adding the four measurements together. Within 24 hours from eclosion, adult weight was measured to the nearest 0.01 mg (Mettler Toledo XPE205 Microbalance, Columbus, OH, USA) and used to calculate wing loading of adult FTC (mg/mm^2).

Statistical analyses

All analyses were conducted on RStudio version 1.0.136 (2018) with a significance level of 0.05 (Table 2-1). Full models included all factors and the interactions between them. Mixed effects models were simplified through removal of non-significant interactions by ANOVA hypothesis testing until the most parsimonious model remained. Plastic cup number, nested within egg mass, nested within origin was used as a random variable. Unless otherwise specified, ANOVA Type II Wald χ^2 tests or ANOVA Type II with Satterthwaite's approximations were used to detect significance. When applicable, Estimated Marginal Means (EMMs) with Tukey method p-value adjustments were conducted as post hoc-tests.

Cocoon Production

Cocoon production, scored as the presence or absence of silk around pupae, was analyzed with a Generalized Linear Mixed-Effects Model (GLMM) for binary outcomes. The independent variables

tested were Diet, Feeding Regime, Origin, and Infection Load, along with their interactions. All insects that reached the pupal stage were used in the model. Overdispersion ($p = 0.891$), Pearson χ^2 Goodness of Fit ($p = 0.959$), and Likelihood Ratio tests indicated the model satisfied the assumptions. Furthermore, a GLMM model for binary outcomes was used to assess the effects of natural infection *versus* inoculation at third instar on cocoon production. Overdispersion ($p = 0.890$), Pearson χ^2 Goodness of Fit ($p = 0.957$), and Likelihood Ratio tests indicated the model satisfied the assumptions.

Cocoon Weight

Given the biological differences between sexes in FTC, cocoon weight was analyzed separately for male and female moths. Due to the low survival ($n = 3$) of female FTC in the partially starved (S) treatment, they were removed from the model. Two Linear Mixed Effect Models (LMMs) with logarithmic transformations were used to explore the effects of Diet, Feeding Regime (only in males), Origin, Infection Load, and their interactions on the total cocoon weight. The models satisfied the assumptions, and the residuals were normally distributed ($p = 0.099$ females, $p = 0.149$ males). Due to the low survival to adult of naturally infected individuals, the difference between inoculated and naturally infected specimens could not be estimated.

Development Time

A LMM with Box-Cox transformation ($\lambda = -0.424$) was used to explore the effects of Diet, Feeding Regime, Origin, Sex, Infection Load, and their interactions on the development time from first instar to adult. An additional LMM with Box-Cox transformation ($\lambda = -0.141$) was used to explore the effects of Diet, Feeding Regime, Origin, Infection Load, and their interactions on the development time from first instar larva to pupal stage, including individuals that died at pupation. A LMM with Box-Cox transformation ($\lambda = 1.515$) also explored the effects of Diet, Feeding Regime, Origin, Sex, and Infection Load on the time spent as a pupa for individuals that eclosed to adults.

To understand the difference in the number of days required to reach pupation between larvae that were naturally infected and those that were infected by inoculation, an additional model was created with the factor “larval infection status” (N = uninfected, Y = infected by inoculation., YY = naturally infected) that replaced infection load.

Adult weight

Given the biological differences between sexes in FTC, body weight was analyzed separately for males and females. Due to the low survival (n = 3) of females in the starved (S) treatment, they were removed from the model. Two LMMs with Box-Cox transformation (males: $\lambda = -0.747$, females: $\lambda = 0.586$) were used to explore the effects of Diet, Feeding Regime (only in males), Origin, Infection Load, and their interactions on adult weight. The models satisfied the assumptions, and the residuals were normally distributed ($p = 0.955$ females, $p = 0.146$ males). Due to the low survival of naturally infected individuals, the difference between inoculated and naturally infected specimens could not be estimated.

Wing Area

Several individuals emerged with malformed wings, which presented as outliers in the data frame. A frequency distribution of the dataset, as well as a visual inspection of specimens, identified malformed wings as a total wing area of $< 190mm^2$ in females and $< 100mm^2$ in males (Appendix D). Individuals with malformed wings were removed from the original dataset.

Wing size was analyzed separately for males and females. Only individuals with normal wings (females $> 190mm^2$, males $> 100mm^2$) were included in the model. Due to the low survival (n = 3) of females in the starved (S) treatment, they were removed from the model. Furthermore, Infection status (N/Y) was used instead of Infection Load, due to the reduced population size in each infection category. Two LMMs were used to explore the effects of Diet, Starvation (males only), Origin, and Infection status on the total wing area. The models passed validation tests, and the residuals were normally distributed.

A GLMM for binary outcomes was used to explore the effects of Diet, Sex, Infection (Y/N), Feeding Regime, and Origin on the prevalence of malformed wings. Overdispersion (p = 0.983), Pearson Chi^2 Goodness of Fit (p=0.511), and Likelihood Ratio tests indicated the model satisfied the assumptions.

Allometric Relationship

Allometric relationships between wing area (mm^2) and adult weight (mg) were assessed to determine whether Diet, Feeding Regime, and Infection Status influenced FTC resource allocation to mobility. Separate LMMs were used to explore the effect of each treatment on male and female FTC moths. Slopes of the regression lines were compared with a t-test to assess whether a treatment effect produced a steeper curve, suggesting allocation preference towards dispersal behaviours (Evenden et al. 2015).

Infection

Infected individuals were separated by infection level as low (1-100 spores) and high (> 100 spores) to determine the effects of Diet, Feeding Regime, Origin, and their interaction on spore replication in FTC. Infection Status (Y/YY) was also included in the model as a fixed factor to determine the difference in infection load among naturally infected (YY) and inoculated at third instar (Y) larvae. All individuals that reached pupation were included in this model. A GLMM for binary outcomes was used. Overdispersion (p = 0.646), Pearson χ^2 Goodness of Fit (p = 0.998), and Likelihood Ratio tests indicated the model fit the data.

Results

Cocoon Production

Infection load significantly affected FTC cocoon production ($\chi^2 = 7.897$, $p = 0.019$). Individuals with a high infection load produced a cocoon in 48.4% of cases (n = 44, N = 91) and had a statistically lower probability of producing a cocoon than uninfected individuals (Z = 2.738, p = 0.017), which produced a cocoon in 66.8% of cases (n = 265, N = 397). Cocoon production by infected individuals with a low spore load occurred in 65.7% of cases (n=23, N=35), which was intermediate between, and did not differ from, that of uninfected individuals (Z = 0.085, p = 0.996) or infected individuals with a high spore load (Z = 1.720, p = 0.198). There was no effect of larval diet ($\chi^2 = 0.021$, $p = 0.884$), feeding regime ($\chi^2 = 1.882$, $p = 0.170$), or egg mass origin ($\chi^2 = 0.003$, $p = 0.955$) on FTC cocoon production (Figure 2-2).

The mode of microsporidia infection impacted cocoon production ($\chi^2 = 13.876$, $p < 0.001$). Naturally infected individuals were less likely to produce a cocoon (6.3%, n = 1, N = 16) than those that were intentionally inoculated with microsporidia (Z = 2.391, p = 0.004) and uninfected (Z = 3.682, p < 0.001) individuals, which produced cocoons in 55.3% (n = 26, N = 47) and 65.6% (n = 107, N = 163) of cases, respectively (Figure 2-3).

Cocoon Weight

Larval diet ($F_{1,83.5} = 0.005$, $p = 0.945$), egg mass origin ($F_{1,43.4} = 1.370$, $p = 0.248$), and infection load ($F_{2,67.0} = 1.071$, $p = 0.344$) did not significantly affect the cocoon weight of female FTC (Figure 2-4).

Egg mass origin ($F_{1,52.0} = 0.107$, $p = 0.745$) and infection load ($F_{2,76.4} = 0.246$, $p = 0.783$) did not significantly affect the cocoon weight of male FTC. Larval diet ($F_{1,72.2} = 6.899$, $p = 0.011$), however, significantly affected cocoon weight of male FTC. Individuals fed on standard diet (SD) had higher cocoon weights compared to individuals fed on standard diet that was supplemented with lyophilized trembling aspen foliage (SDA). Cocoon weight was also significantly affected by the larval feeding regime

($F_{1,78.7}$ = 20.828, p < 0.001). Full regime moths produced more silk compared to partially starved individuals (Figure 2-5).

Development Time

Larvae fed the standard diet (SD) reached adult eclosion faster than larvae fed the standard diet supplemented with lyophilized trembling aspen foliage (SDA) ($F_{1,93.6}$ = 10.584, p = 0.002). Partially starved larvae (S) took significantly longer than full regime larvae (F) to complete development to adult eclosion ($F_{1,112.4}$ = 51.634, p < 0.001). Male FTC eclosed before females ($F_{1,266.5}$ = 8.934, p = 0.003). Individuals that were reared from egg masses collected in Alberta took developed faster to adults than individuals reared from egg masses collected in Ontario ($F_{1,66.3}$ = 8.270 p = 0.005). Infection load ($F_{2,280.6}$ = 0.152, p = 0.859) did not significantly affect FTC time to eclosion (Figure 2-6). The way in which larvae were infected, either naturally or through inoculation, did not affect development time to adult ($F_{2,96.9}$ = 2.005, p = 0.140).

Development time from first instar larvae to pupae was significantly affected by larval diet ($F_{1,88.5}$ = 26.486, p < 0.001), feeding regime ($F_{1,122.0}$ = 75.763, p < 0.001), and egg mass origin ($F_{1,77.0}$ = 14.425, p < 0.001). Larvae reared on the standard diet (SD) developed significantly faster than those reared on the standard diet supplemented with trembling aspen foliage (SDA). Larvae fed the full regime (F) reached the pupal stage faster than partially starved (S) larvae. Larvae that originated from egg masses collected in Alberta developed significantly faster than larvae that originated from Ontario egg masses. Infection load did not significantly affect development time to the pupal stage ($F_{2,183.8}$ = 2.298, p = 0.103, Figure 2-7).

Larval diet ($F_{1,234.0}$ = 5.082, p = 0.025), sex ($F_{1,288.1}$ = 9.488, p = 0.002), and egg mass origin ($F_{1,63.4}$ = 5.241, p = 0.025) significantly affected the length of the pupal stage. Insects reared on the standard diet (SD) spent less time in the pupal stage than those reared on the diet supplemented with trembling

aspen foliage (SDA). The pupal stage of female FTC was shorter than that of males. Lastly, the pupal stage was shorter in individuals that originated from egg masses collected in Alberta than those from Ontario. Feeding regime ($F_{1,273.9} = 0.075$, $p = 0.784$) and infection load ($F_{2,280.6} = 0.152$, $p = 0.859$) did not significantly affect the duration of the pupal stage (Figure 2-8).

Adult Weight

The origin of the egg mass ($F_{1,37.4} = 8.215$, $p = 0.007$) significantly affected the adult body weight of female FTC. Female moths that were reared from egg masses that originated from Ontario were heavier than females from egg masses collected in Alberta. Larval diet ($F_{1,92.1} = 1.202$, $p = 0.276$) and infection load ($F_{2,81.5} = 0.094$, $p = 0.910$) did not significantly affect the adult body weight of female FTC (Figure 2-9).

Larval diet ($F_{1,160.7} = 5.789$, $p = 0.017$), feeding regime ($F_{1,125.0} = 6.406$, $p = 0.013$), and egg mass origin ($F_{1,52.6} = 4.038$, $p = 0.050$) statistically influenced adult male body weight. Larvae fed on the standard diet supplemented with trembling aspen foliage (SDA) developed into heavier male moths compared to larvae fed on the standard diet (SD). Male moths fed the full regime (F) were significantly heavier than partially starved (S) males. Male moths reared from egg masses that originated in Ontario were heavier than male moths obtained from egg masses collected in Alberta. Lastly, even though infection load ($F_{2,155.4} = 2.152$, $p = 0.120$) did not significantly affect male moth weight, a clear trend was visible. Males with high infection load were heavier ($\bar{x} = 22.523 \pm 11.213$ mg) than those with a low infection load ($\bar{x} = 20.823 \pm 5.918$ mg), and uninfected males were the lightest ($\bar{x} = 19.570 \pm 7.640$ mg) (Figure 2-10).

Wing Area

Larval diet affected wing area in female moths ($F_{1,100} = 4.671$, $p = 0.033$), but not in males ($F_{1,126.2} = 2.191$, $p = 0.141$). Female larvae fed the standard diet (SD) had significantly larger wings than females

fed the standard diet supplemented with lyophilized trembling aspen foliage (SDA). Egg mass origin did not significantly affect wing area in either female ($F_{1,36.6} = 2.840$, $p = 0.100$) or male ($F_{1,46.2} = 0.469$, $p = 0.497$) moths. Similarly, microsporidian infection did not influence the wing area of female ($F_{1,92.5} = 0.048$, $p = 0.828$) or male ($F_{1,128.2} = 3.388$, $p = 0.068$) moths. Male larvae fed the full regime (F) had significantly larger wings than partially starved males (S) ($F_{1,126.7} = 8.057$, $p = 0.005$) (Figure 2-11, Figure 2-12).

Larval diet ($\chi^2 = 9.361$, $p = 0.002$) significantly affected the probability that adult FTC would eclose with malformed wings. Larvae reared on the standard diet (SD) developed malformed wings in 7.4% of cases (n = 17, N = 229), significantly less likely than moths that developed from larvae fed on the standard diet supplemented with lyophilized trembling aspen foliage (SDA), which displayed wing malformations in 25.0% of cases (n = 8, N = 32). Infection status ($\chi^2 = 0.501$, $p = 0.479$), egg mass origin ($\chi^2 = 0.743$, $p = 0.389$), sex ($\chi^2 = 0.004$, $p = 0.949$), and feeding regime ($\chi^2 = 0.410$, $p = 0.522$) did not significantly affect the proportion of moths that eclosed with malformed wings.

Allometric relationship (mg/mm^2)

The allometric relationship between bodyweight and wing size was positive for male ($t_{117.6} = 2.798$, $p = 0.006$) and female ($t_{99.8} = 4.203$, $p < 0.001$) moths reared on SD. On the contrary, standard diet supplemented with 1% lyophilized trembling aspen foliage (SDA) altered the allometric relationship, resulting in a non-significant relationship between wing area (mm^2) and adult weight (mg) in both male ($t_{122.1} = -0.241$, $p = 0.810$) and female ($t_{95.2} = -0.939$, $p = 0.350$) moths. Given the absence of an allometric relationship in SDA, the slopes of the two diet treatments could not be compared. Similarly, uninfected male ($t_{116.3} = 2.258$, $p = 0.026$) and female ($t_{99.5} = 3.201$, $p = 0.002$) moths had a positive relationship between body mass and wing area, contrary to infected male ($t_{124.8} = -0.181$, $p = 0.857$) and female ($t_{99.5} = 1.739$, $p = 0.085$) moths, which had non-significant slopes. Partial starvation caused a loss of the allometric relationship ($t_{125} = -0.785$, $p = 0.434$) in males, but not in the full regime treatment, which

presented a positive relationship between bodyweight and wing area ($t_{120.8}$ = 2.181, p = 0.031). Both male ($t_{107.8}$ = 2.004, p = 0.043) and female ($t_{99.5}$ = 2.232, p = 0.028) moths from Ontario had a significant relationship between body mass and wing area. This was also true for female moths from Alberta ($t_{99.2}$ = 2.805, p = 0.006), but not for males ($t_{123.8}$ = 1.157, p = 0.250). The slopes of the relationships in Alberta and Ontario did not differ for female moths ($t_{98.7}$ = 0.498, p = 0.620) (Figure 2-13, Figure 2-14).

Infection

Infection load was not significantly affected by larval diet (χ^2 = 0.127, p = 0.910), feeding regime (χ^2 = 0.002, p = 0.969), or origin (χ^2 = 0.047, p = 0.828). On the other hand, naturally infected FTC were significantly more likely to be heavily infected than larvae inoculated at third instar (χ^2 = 4.862, p = 0.028) (Figure 2-15).

Discussion

Our experiment did not support the prediction that infection with a microsporidian parasite interacts with qualitative and quantitative properties of larval diet to affect FTC growth and development. There was no significant interaction among infection and the qualitative or quantitative treatments of larval diet on any of the parameters measured in this study. Moreover, alterations of larval diet and feeding regime did not impact spore replication within the host. Larval diet and entomopathogen infection interact to affect the growth and development of several insect species (eg: Cook et al., 2003; Duffey et al., 1995; Hoover et al., 1998; James & Lighthart, 1992; Steele & Bjørnson, 2019). Susceptibility to entomopathogens, including microsporidia, can be affected by food quality (Carloye et al., 1998; Gómez-Moracho et al., 2021) and food limitation (Steele & Bjørnson, 2019; Steele et al., 2020), but the exact mechanisms are largely unknown. For example, the lethal concentration of the entomopathogen *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) is lower when young larvae

of the corn earworm *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), southern armyworm *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae), and soybean looper *Pseudoplusia includens* Walker (Lepidoptera: Noctuidae) feed on cotton compared to soybean and tomato leaves (Ali et al., 2004). Moreover, nutritional stress is correlated with increased infectivity of *Nosema adaliae* (Dissociodihaplophasida: Nosematidae) in *Adalia bipunctata* Linneaus (Coleoptera: Coccinellidae) (Steele et al., 2020). Gallotannin in red oak *Quercus rubra* Linneaus (Fagales: Fagaceae) is linked to reduced pathogenicity of the nucleopolyhedrovirus LdNPV in gypsy moths *Lymantria dispar* Linneaus (Lepidoptera: Erebidae) (Hunter & Schultz, 1993). In FTC, studies on this tritrophic interaction are limited. Infection with *B. thuringiensis* (variety *kurstaki* HD-1) is less lethal by over 100 times for FTC larvae fed on trembling aspen as compared to sugar maple (Kouassi et al., 2001). On the contrary, FTC reared on sugar maple or trembling aspen respond in the same manner to microsporidian infection (Chapter 3).

Research on entomopathogenic microsporidia in Lepidoptera is limited, likely due to their restricted potential as biological control agents (Canning, 1982). Microsporidia contribute, however, to natural control of populations in a wide array of Lepidoptera (Maddox et al., 1998), and may, therefore, offer important insights on insect cyclical population dynamics. The impact of larval diet on the pathogenicity of infection by microsporidia in Lepidoptera is variable, and the exact mechanisms of these interactions are mostly unknown. For example, mortality of the cabbage looper *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae) by *Vairimorpha* (Dissociodihaplophasida: Nosematidae) infection is significantly delayed when the allelochemical xanthotoxin is incorporated into the larval diet (Carloye et al., 1998). On the contrary, pathogenicity of *Nosema fumiferanae* (Dissociodihaplophasida: Nosematidae) to the spruce budworm, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae), is not altered when larvae feed on previous year foliage, an inferior source of nutrition (van Frankenhuyzen & Liu, 2016). Furthermore, cabbage looper sensitivity to infection to a *Vairimorpha* sp. is

not altered when fed onion extract (Carloye et al., 1998), a plant extract that is able to inhibit microsporidian infection in the ugly-nest caterpillar, *Archips cerasivorana* Fitch (Lepidoptera: Tortricidae) (Smirnoff, 1967). These results show the equivocal relationship between larval diet and microsporidian infection in various Lepidoptera species. In FTC, host affiliation does not interact with pathogenicity of microsporidian infection (Chapter 3). This is in agreement with our experiment, which demonstrates that incorporation of lyophilized trembling aspen foliage into a synthetic diet does not result in a significant diet by disease interaction. It is important to notice, however, the population size of infected individuals (Y) reared on standard diet supplemented with 1% lyophilized trembling aspen foliage (SDA) that survived to adulthood was extremely low (n = 5), likely due to the additive negative effects of a suboptimal diet and microsporidian infection (Thomsom 1959). The same consideration can be made for infected individuals that were partially starved and reached adulthood (n = 4). Our study focused on adult life history traits and did not investigate the possible implications of this tritrophic interaction within larval mortality. It is therefore possible microsporidian infection and larval diet interact to increase larval mortality in FTC.

Effects of microsporidian infection can change depending on the concentration and timing of infection (Wilson, 1984), which might explain the different results obtained for naturally infected and inoculated FTC. Even though we did not run molecular analyses on the microsporidia extracted from FTC larvae in our study, we assume the infection is primarily a result of microsporidia in the genus *Nosema*, based on the size of the spores (4.3 x 2.2 µm, Appendix E) and the regularity of infection by this species in FTC (Sohi & Wilson, 1976; Thomson, 1958). We hypothesize it is the species first identified as *Perezia disstriae* (Thomson, 1959) and later reclassified as *Nosema disstriae* (Kyei-Poku & Sokolova, 2017). *N. disstriae* infection in FTC is associated with heavy spore concentration in the silk glands (Thomson, 1959), which may lead to the absence of a cocoon around the pupa (Wilson, 1977). Similar patterns of *Nosema* infection occur in several lepidopteran species. For example, spores of *N. lymantriae* and *N.*

serbica accumulate in salivary glands of gypsy moths (Weiser, 1998), *N. tyriae* in the cinnabar moth *Tyria jacobaeae* Linneaus (Lepidoptera: Erebidae) (Canning et al., 1999), *N. furmacalis* in the asian corn borer *Ostrinia furnacalis* Guenée (Lepidoptera: Crambidae) (Iwano & Kurtti, 1995), and *N. bombycis* in the domestic silk moth *Bombyx mori* Linneaus (Lepidoptera: Bombycidae) (Ishihara, 1968). Our study did not look directly at microsporidian infection load in the FTC silk glands; however, highly infected larvae were significantly less likely to produce silken cocoons, confirming previous findings (Wilson, 1977). Naturally infected individuals had a lower probability of cocoon production and a higher spore count than larvae inoculated at third instar. This suggests the timing of infection plays a major role in the pathogenicity of microsporidian infection in FTC (Wilson, 1977). There was no significant difference in the probability of cocoon production between uninfected larvae and larvae with a low spore load, suggesting that high spore loads are required to functionally reduce silk production. Alternatively, infection may create a threshold response leading to a complete loss of gland function. Interestingly, there was no effect of microsporidia on cocoon weight when silk was produced. This may be linked to the high concentration of spores in the silk (Thomson, 1959), which may have added supplemental weight to the cocoons. Given juvenile FTC use silk trails to move gregariously in search of food (Fitzgerald & Costa, 1986), future studies should explore the potential effects of high microsporidian infection in larval silk glands. A disruption of their communication system may lead to detrimental consequences in juvenile foraging ability, which would inevitably alter population dynamics of this gregarious insect.

Resource allocation represents the correlation of energy obtained by foraging and allocation of that energy to life history strategies (Boggs, 1992). Qualitative and/or quantitative nutritional uptake can impact resource allocation to adult life history traits in holometabolous insects (Angelo & Slansky, 1984; Coll & Yuval, 2004), especially when all of the feeding occurs as larvae (Jervis et al., 2005), as is the case for FTC. For example, fluctuations in FTC population density affects resource allocation to flight and reproduction in adult moths (Evenden et al., 2015a; Jones & Evenden, 2008), including pheromone-

mediated mating behaviors (Evenden et al., 2015b). Moreover, changes in carbohydrate to protein ratios in larval diet can severely alter FTC resource allocation towards somatic or reproductive growth (Colasurdo et al., 2009). Carbohydrate rich diets favour somatic growth over ovary growth, therefore reducing reproductive potential. On the contrary, FTC reared on high protein diets, or C:P balanced diets, allocate more resources towards reproduction. Our results support these findings and suggest that changes in quality and/or quantity of larval diet are correlated with alterations in resource allocation. Partial starvation resulted in smaller wings and the complete loss of any allometric relationship between wing area and body weight. Similar findings have been reported in other Lepidoptera (Boggs & Ross, 1993; Johnson et al., 2014; Ruohomäki, 1992). For example, partial starvation of the mormon fritillary, *Speyeria mormonia* Edwards (Lepidoptera: Nymphalidae), suggests Lepidoptera may resorb and reallocate resources under nutritional stress (Boggs & Ross, 1993). In our study, the allometric relationship was lost even when standard diet was supplemented with 1% lyophilized trembling aspen. The slope of the allometric relationship in both male and female moths was significantly steeper when reared on SD compared to SDA. This would usually be attributed to a promotion in reproductive potential (Evenden et al. 2015), however, given the lack of an allometric relationship between wing area and body weight in moths reared on SDA, we cannot confirm a diet induced switch in the evolutionary trade-off. This suggests, however, that plant phytochemistry plays a major role in shaping FTC life history strategies, as observed in other Lepidoptera (Mozaffarian et al., 2007; Soule et al., 2020). For example, the monarch butterfly develops wider and shorter wings when larval foraging occurs on tropical milkweed, *Asclepias curassavica* Linnaeus (Gentianales: Apocynaceae), suggesting an evolutionary trade-off in favour of reproduction (Soule et al., 2020). On the contrary, larvae reared on swamp milkweed, *Asclepias incarnata* Linnaeus (Gentianales: Apocynaceae), or common milkweed, *Asclepias syriaca* Lineaus (Gentianales: Apocynaceae), are characterized by phenotypic traits more commonly associated with dispersal. Microsporidian infection was also linked to

a complete loss of allometric relationship between wing area and body weight in our study, confirming previous work on other forest Lepidoptera (Eveleigh et al. 2007). Microsporidian infection can interfere with body size-fecundity relationships (Evenden et al., 2006), and can reduce wing area in Lepidoptera (Chapter 3; Zhang et al., 2012). Our results support these earlier findings and suggest microsporidian infection may interfere with the evolutionary trade-off between dispersal and reproduction in FTC.

In our experiment, significant alterations of FTC adult life history traits were linked to manipulations of larval diet. This study was based on the knowledge that increased population density during outbreaks may lead to intraspecific competition of larvae, resulting in reduced food quantity and/or quality (Fitzgerald, 1995; Sutton & Tardif, 2007). Starvation has been attributed as a major factor shaping outbreaks duration in forest Lepidoptera (Abbott & Dwyer, 2007), and has been associated with population collapses in FTC (Fitzgerald, 1995). Laboratory experiments on partial larval starvation show that pupal mass is negatively affected (Evenden et al. 2015b) and mortality rates are higher (Hodson, 1941). Complete starvation of first (Smith & Raske, 1968) and fifth (Hodson, 1941) instar FTC larvae decrease larval survival. Furthermore, adults that develop from nutritionally stressed larvae are smaller and less fecund (Hodson, 1941). Nutritional stress has also been linked with alterations in resource allocations to reproduction and dispersal, as observed in our study. For example, nutritionally stressed autumnal moths *Epirrita autumnata* Borkhausen (Lepidoptera: Geometridae) have smaller wings in relation to body size than well fed insects (Ruohomäki, 1992). In addition, nutritionally stressed Lepidoptera may be able to produce offspring with higher resistance to starvation (Carisey & Bauce, 2002). For these reasons, it is important to explore how limited food availability can affect population dynamics and adult life history traits of forest defoliators, both in the short and long term. From our results, we can infer that partial starvation and microsporidian infection do not interact to impact the adult life history traits measured in this study, instead, they likely work in an additive fashion that may contribute to population cycling of FTC.

Supplementation of the standard diet with 1% lyophilized trembling aspen detrimentally affected FTC growth and development. The addition of trembling aspen to the diet resulted in lower cocoon weight (males), smaller wing area (females), a higher probability of wing malformation, and delayed overall development time of FTC. Furthermore, the standard diet modified with trembling aspen caused drastic changes in the allometric relationship between adult weight (mg) and wing area (mm^2) in both male and female moths. It is important to notice, however, that the incorporation of trembling aspen into the larval diet resulted in an increase in body weight in male moths, likely as result of prolonged development time. Forest tent caterpillars are affected by changes in both primary and secondary metabolites when feeding on trembling aspen or artificial diets (Donaldson & Lindroth, 2008; Hemming & Lindroth, 1995; Lindroth & Bloomer, 1991; Noseworthy & Despland, 2006). Given the low percentage (1%) of trembling aspen foliage used in our modified diet, it is unlikely that variation of primary metabolites is playing a major role in the observed differences. Therefore, detrimental effects of trembling aspen supplementation are likely attributed to secondary metabolites in the dried plant tissue. Trembling aspen has an array of secondary metabolites, including phenolic compounds such as phenolic glycosides and condensed tannins, that are associated with chemical defense against herbivory (Lindroth et al., 1987; Palo, 1984). Concentrations of these secondary metabolites vary depending on tree age, genetics, and through changes in biotic and abiotic factors (eg: Clausen, 1991; Donaldson & Lindroth, 2007; Erwin et al., 2001; Hemming & Lindroth, 1995; Hemming & Lindroth, 1999; Hwang & Lindroth, 1997; Lindroth & Hwang, 1996; Osier et al., 2000; Osier & Lindroth, 2001). Among phenolic glycosides, the most common compounds in trembling aspen are salicin, salicortin, tremuloidin, and tremulacin, which usually occur in concentrations between 1 – 8% of leaf dry weight (Lindroth & Hwang, 1996; Osier et al., 2000). The concentrations of condensed tannins are more variable, but can amount to nearly 30% of leaf dry weight (Lindroth & Hwang, 1996; Osier et al., 2000). Previous studies on trembling aspen chemical defense in response to lepidopteran herbivory indicate a clear relationship between

levels of phenolic compounds and larval performance. Chemical variation both among trembling aspen clones and within individual trees impact herbivore success (Chilcote et al., 1992; Donaldson & Lindroth, 2007; Lindroth & Hwang, 1996; Meyer & Montgomery, 1987; Osier et al., 2000) and can lead to detrimental effects in both adapted (Chilcote et al., 1992; Hemming & Lindroth, 1995; Hough & Pimentel, 1978; Hwang & Lindroth, 1997; Lindroth et al.,1991) and unadapted lepidopteran species (Lindroth & Peterson, 1988; Lindroth et al., 1988). For example, larvae of the aspen-unadapted southern armyworm fed salicortin and tremulacin isolated from trembling aspen had reduced growth rates and lesions in the midgut tissue (Lindroth & Peterson, 1988). Aspen-adapted forest Lepidoptera, such as FTC and gypsy moth, are also impacted by secondary metabolites in trembling aspen. Phytochemicals in trembling aspen foliage are directly linked to detrimental effects on survival, growth rate, food consumption, approximate digestibility, and nutrient processing efficiency in these two species (Chilcote et al., 1992; Hemming & Lindroth, 1995; Hwang & Lindroth, 1997; Jocelyn & Lindroth, 2000; Lindroth & Hemming, 1990; Lindroth & Bloomer, 1991; Lindroth et al., 1991; Lindroth & Weisbrod, 1991). For example, FTC and gypsy moth development is slowed and the growth rate is reduced when 4th instar larvae are reared on trembling aspen leaves supplemented with 4% phenolic glycosides (Jocelyn & Lindroth, 2000). These results are in line with our observations, which demonstrated artificial diet supplemented with 1% lyophilized trembling aspen foliage hindered FTC fitness. This is likely attributed to a diminished efficiency in food conversion by FTC, which resulted in a reduced nutrient uptake. The freeze-drying process is known to retain higher concentrations of phenolic compounds (Coklar et al., 2018; Meng et al., 2018; Saifullah et al., 2019). Moreover, young leaves were used in the study, which are characterized by higher concentrations of phenolic compounds than older trembling aspen leaves (Lindroth & Weisbrod, 1991). Freeze-drying aspen foliage may have concentrated phenolic compounds such as phenolic glycosides and condensed tannins in the diet, causing the observed detrimental effects in FTC.

We used larvae that originated from both Alberta and Ontario in our experiment. Larvae reared from Ontario egg masses outperformed FTC from Alberta in adult weight but required a longer development time. The Alberta egg masses were collected from Lac La Biche in the winter of 2018-2019, following high population density in 2017 and 2018 (Appendix A, Appendix B). This might have influenced larval feeding of parental moths due to intraspecific competition (Fitzgerald, 1995), resulting in reduced fecundity (Hodson, 1941) and, potentially, hindered offspring fitness. Ontario FTC resulted in a higher concentration of spore load. Interestingly, our experiment from 2014 also reported higher spore concentrations in Ontario than Alberta (Chapter 3). This may suggest geographic variation in microsporidian infection in controlling population dynamics of FTC. Future studies should explore microsporidia abundance and identity throughout the geographical range of FTC. This may allow us to better understand the driving forces behind differences in the population cycles of Eastern and Western FTC populations (Cooke & Roland, 2018).

Tables

Table 2-1. List of models used for statistical analyses. Diet (SD = standard diet, SDA = standard diet + trembling aspen), Feeding Regime (F = full regime, S = starved), Origin (Alberta, Ontario), Sex (M = Males, F = Females), and Infection Load (None: 0 spores, Low: 1-100 spores, High: 101+ spores count per 10 fields of view at 400x magnification).

Response (Dataset)	**Model**
Cocoon Production (All data)	glmer (Cocoon ~ Diet + Feeding Regime + Origin + Infection Load, random = (origin/eggmass/cup)
Cocoon Production (Larval Infection)	glmer (Cocoon ~ Diet + Feeding Regime + Origin + Infection Status, random = (origin/eggmass/cup)
Cocoon Weight (Females)	lmer (log(Cocoon Weight) ~ Diet + Infection Load + Origin, random = (origin/eggmass/cup)
Cocoon Weight (Males)	lmer (log(Cocoon Weight) ~ Diet + Infection Load + Feeding Regime + Origin, random = (origin/eggmass/cup)
Time to Pupation	lmer $(Pupae)^{-0.141}$ ~ Diet + Feeding Regime + Origin + Infection Load, random = (origin/eggmass/cup)
Pupation Time	lmer $(Pupation)^{-1.515}$ ~ Diet + Feeding Regime + Origin + Infection Load + Sex, random = (origin/eggmass/cup)
Eclosion Tme	lmer $(Adults)^{-0.424}$ ~ Diet + Feeding Regime + Origin + Infection Load + Sex, random = (origin/eggmass/cup)
Adult Weight (Females)	lmer$(Weight)^{0.586}$ ~ Diet + Origin + Infection Load, random = (origin/eggmass/cup)
Adult Weight (Males)	lmer$(Weight)^{-0.747}$ ~ Diet + Origin + Feeding Regime + Infection Load, random = (origin/eggmass/cup)
Wing Area (Females)	lmer((Wings) ~ Diet + Infection + Origin, random = (origin/eggmass/cup)
Wing Area (Males)	lmer((Wings) ~ Diet + Infection + Origin + Feeding Regime, random = (origin/eggmass/cup)
Malformed Wings	glmer(Malformed ~ Diet + Infection + Origin + Sex + Feeding Regime, random = (origin/eggmass/cup)
Allometric Relationship	glmer(Weight ~ Treatment/Wings, random = (origin/eggmass/cup)
Infection Load	glmer(Infection Load ~ Diet + Origin + Feeding Regime + Infection Status, random = (origin/eggmass/cup)

Table 2-2 Population sizes (N) used for each analysis divided by Diet (SD = standard diet, SDA = standard diet + trembling aspen), Feeding Regime (F = full regime, S = partially starved), Infection Load (N: 0 spores, Low: 1-100 spores, High: 101+ spores count per 10 fields of view at 400x magnification), and Origin (Alberta, Ontario).

	Diet		**Feeding Regime**		**Infection**			**Origin**	
Analysis	**SD**	**SDA**	**F**	**S**	**N**	**Y**		**Alberta**	**Ontario**
Cocoon Production	456	67	496	27	397	Low: 35	High: 91	253	270
Cocoon Weight (females)	77	9	86	0	69	Low: 6	High: 11	46	40
Cocoon Weight (males)	91	17	96	12	87	Low: 9	High: 12	49	59
Time to Pupation	456	67	496	27	397	Low: 35	High: 91	253	270
Pupation Time	258	39	280	17	234	Low: 18	High: 45	145	152
Eclosion Time	258	39	280	17	234	Low: 18	High: 45	145	152
Weight (females)	110	14	121	3	96	Low: 6	High: 22	66	58
Weight (males)	148	25	159	14	138	Low: 12	High: 23	79	94
Wing Area (females)	96	8	104	0	77	27		54	50
Wing Area (males)	113	16	119	10	102	27		56	73
Wing Malformation	229	32	246	15	200	61		124	137
Allometric Relationship (females)	96	8	104	0	77	27		54	50
Allometric Relationship (males)	113	16	119	10	102	27		56	73

Figures

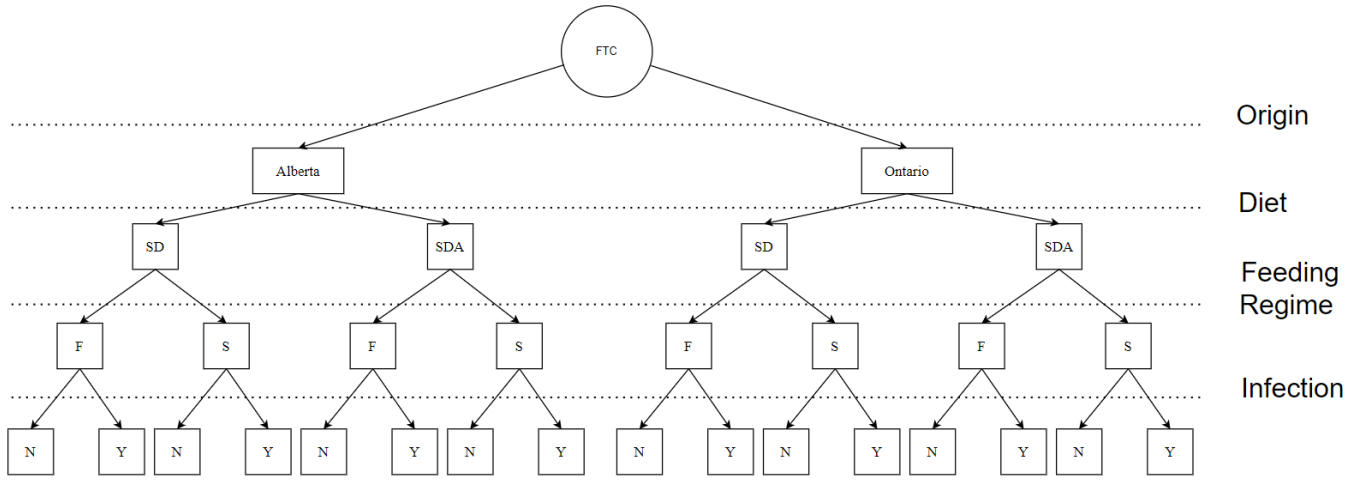

Figure 2-1. Experimental design of the treatments tested. Based on Origin: Alberta & Ontario, Diet: SD = Standard diet, SDA = Standard diet + 1% lyophilized trembling aspen foliage, Feeding Regime: F = Full regime, S = Partially starved, and Infection: N = Uninfected, Y = Infected.

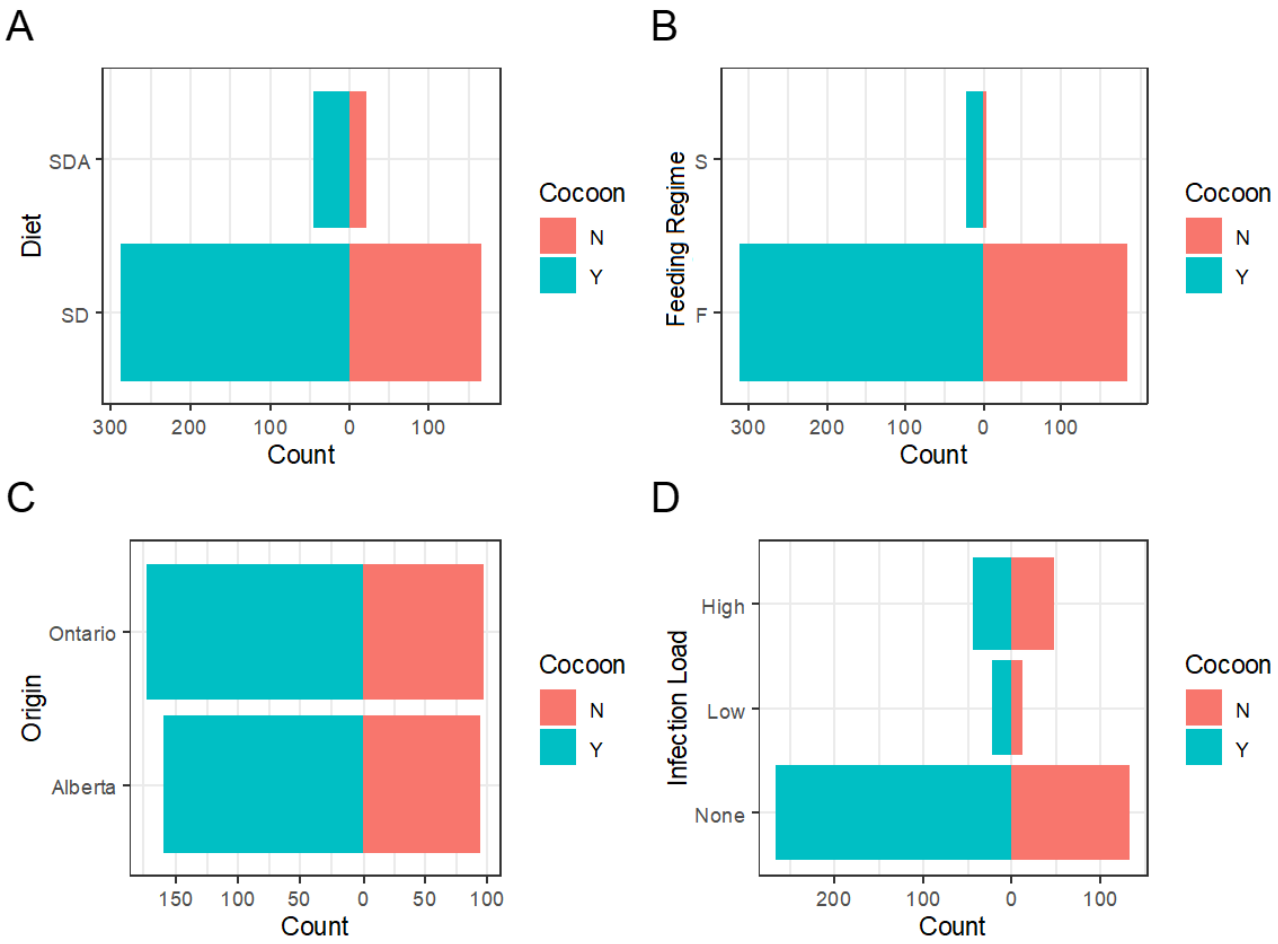

Figure 2-2. Pyramid plots of cocoon production (N = no cocoon, Y = cocoon present) of all FTC that reached pupation. (A) Diet: SD = Standard diet, SDA = Standard diet + 1% lyophilized trembling aspen foliage, (B) Feeding Regime: F = Full regime, S = Partially starved, (C) Origin: Alberta & Ontario, and (D) Infection Load: None = 0 spores, Low = 1 < spores < 100, High = 100+ spores per 10 fields of view at 400x magnification.

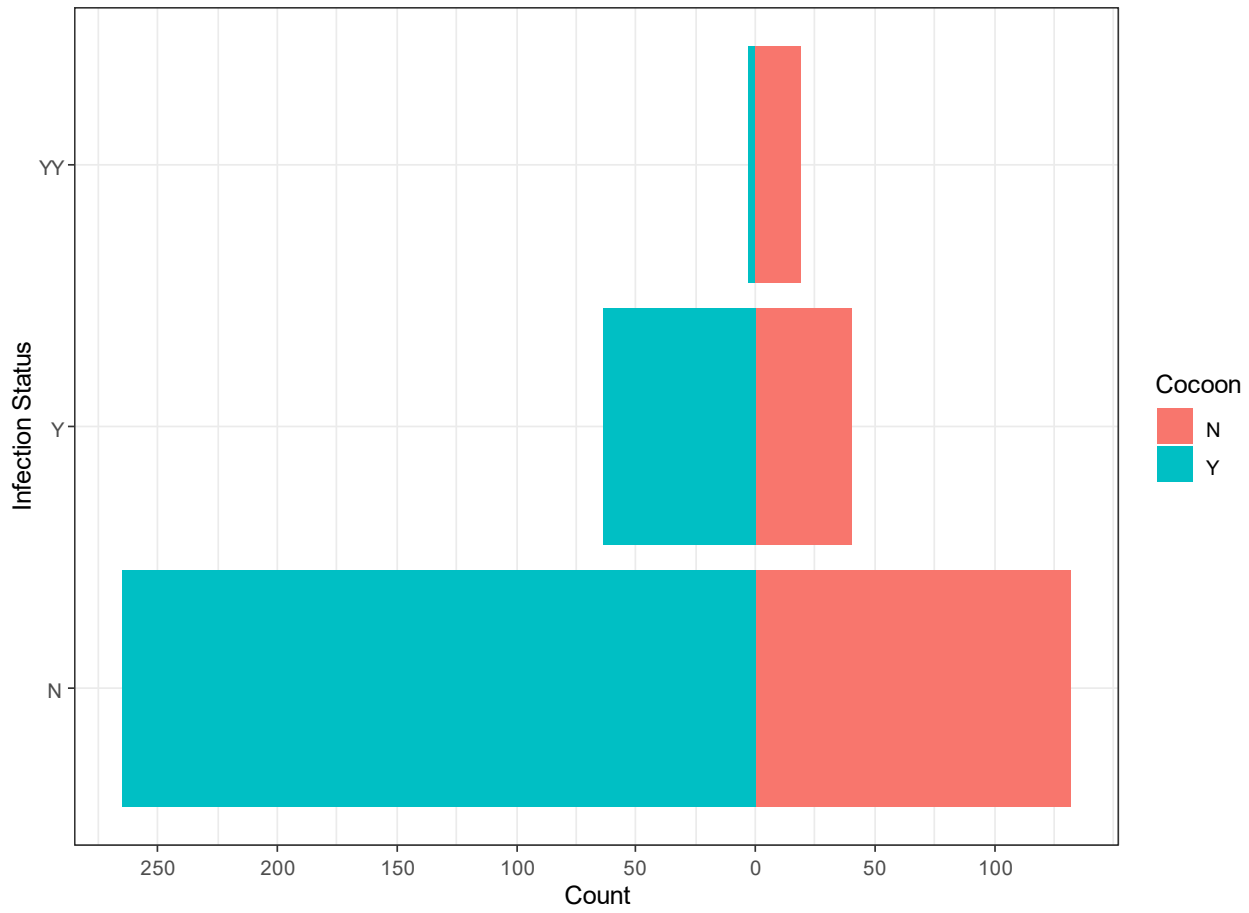

Figure 2-3. Pyramid plots of cocoon production (N = no cocoon, Y = cocoon present) of all FTC that reached pupation by larval infection status: N = uninfected, Y = infected by inoculation, YY = infected naturally.

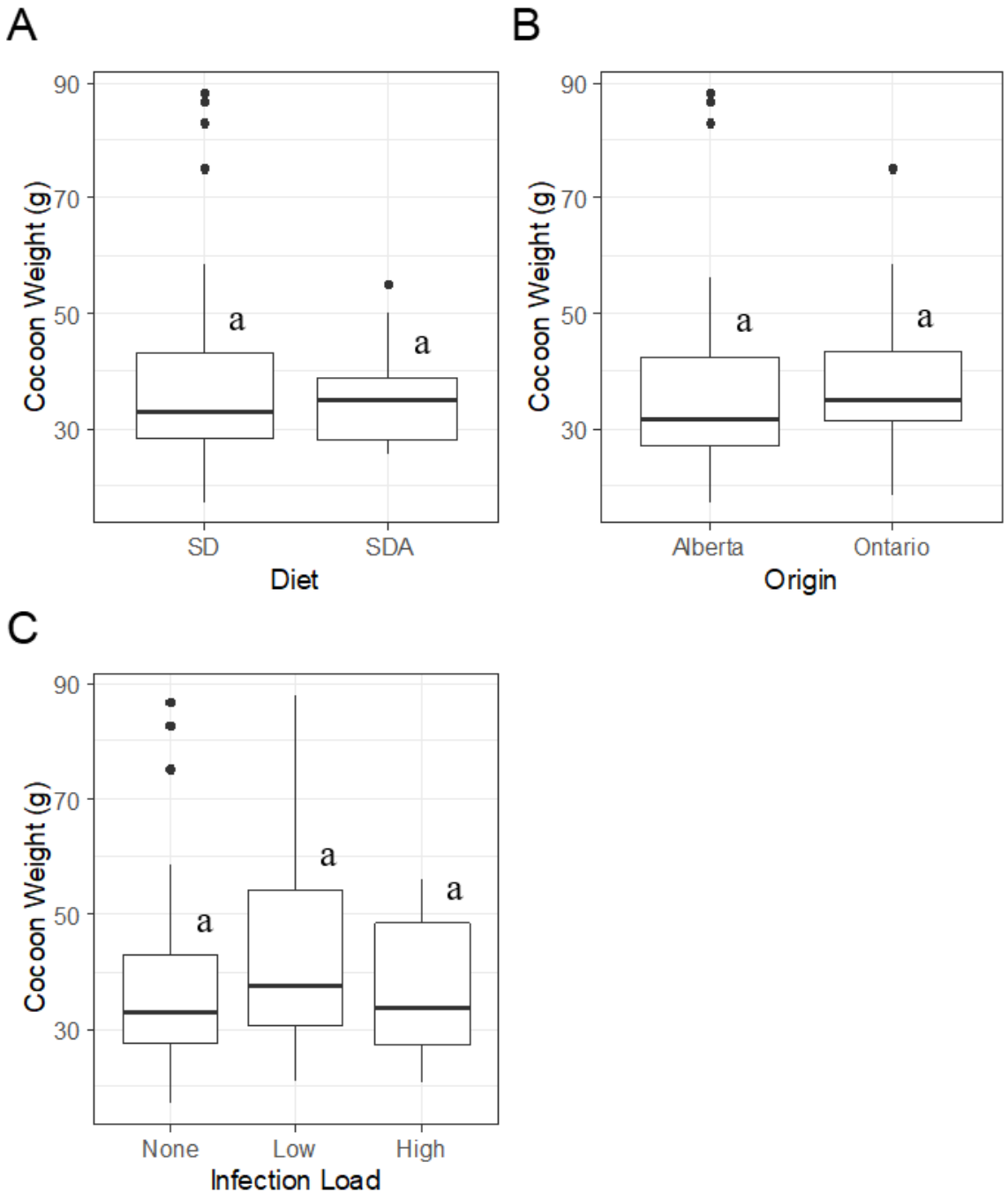

Figure 2-4. Boxplots of Cocoon Weight (mg) of female FTC by (A) Diet: SD = Standard Diet, SDA = Standard diet + trembling aspen foliage, (B) Origin: Alberta & Ontario, and (C) Infection Load: None = 0 spores, Low = 1 < spores < 100, High = 100+ spores per 10 fields of view at 400x magnification. The midline of each box represents the median, whereas the bottom and top of each box indicates the first and third quartiles, respectively. The whiskers extending from each box indicate the maximum and minimum values and the black dots are outliers. Boxplots marked with different letters are significantly different (t test, P < 0.05).

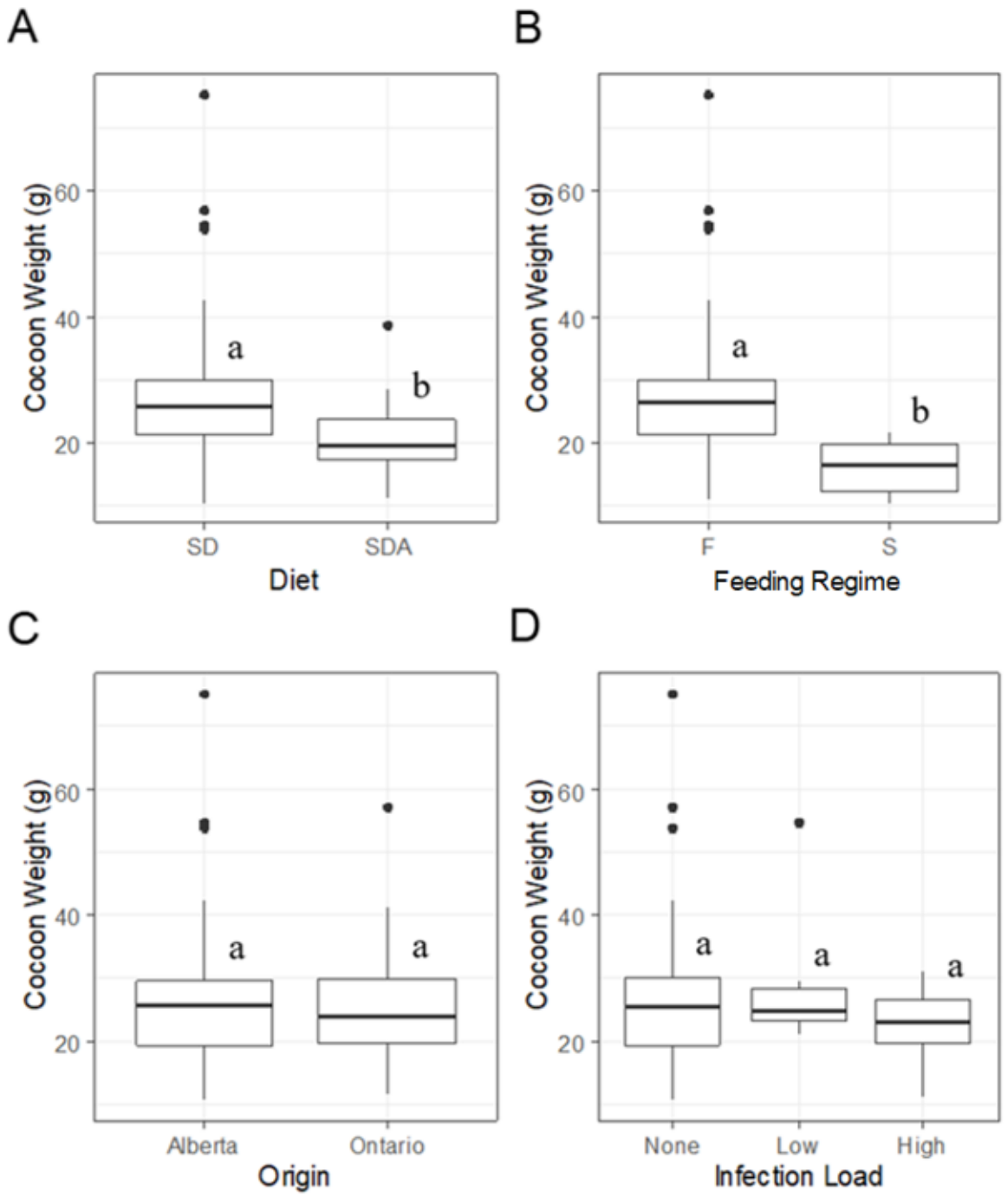

Figure 2-5. Boxplots of Cocoon Weight (mg) of male FTC by (A) Diet: SD = Standard Diet, SDA = Standard Diet + trembling aspen foliage, (B) Feeding Regime: F = Full regime, S = Starved, (C) Origin: Alberta & Ontario, and (D) Infection Load: None = 0 spores, Low = 1 < spores < 100, High = 100+ spores per 10 fields of view at 400x magnification. The midline of each box represents the median, whereas the bottom and top of each box indicates the first and third quartiles, respectively. The whiskers extending from each box indicate the maximum and minimum values and the black dots are outliers. Boxplots marked with different letters are significantly different (t test, $P < 0.05$).

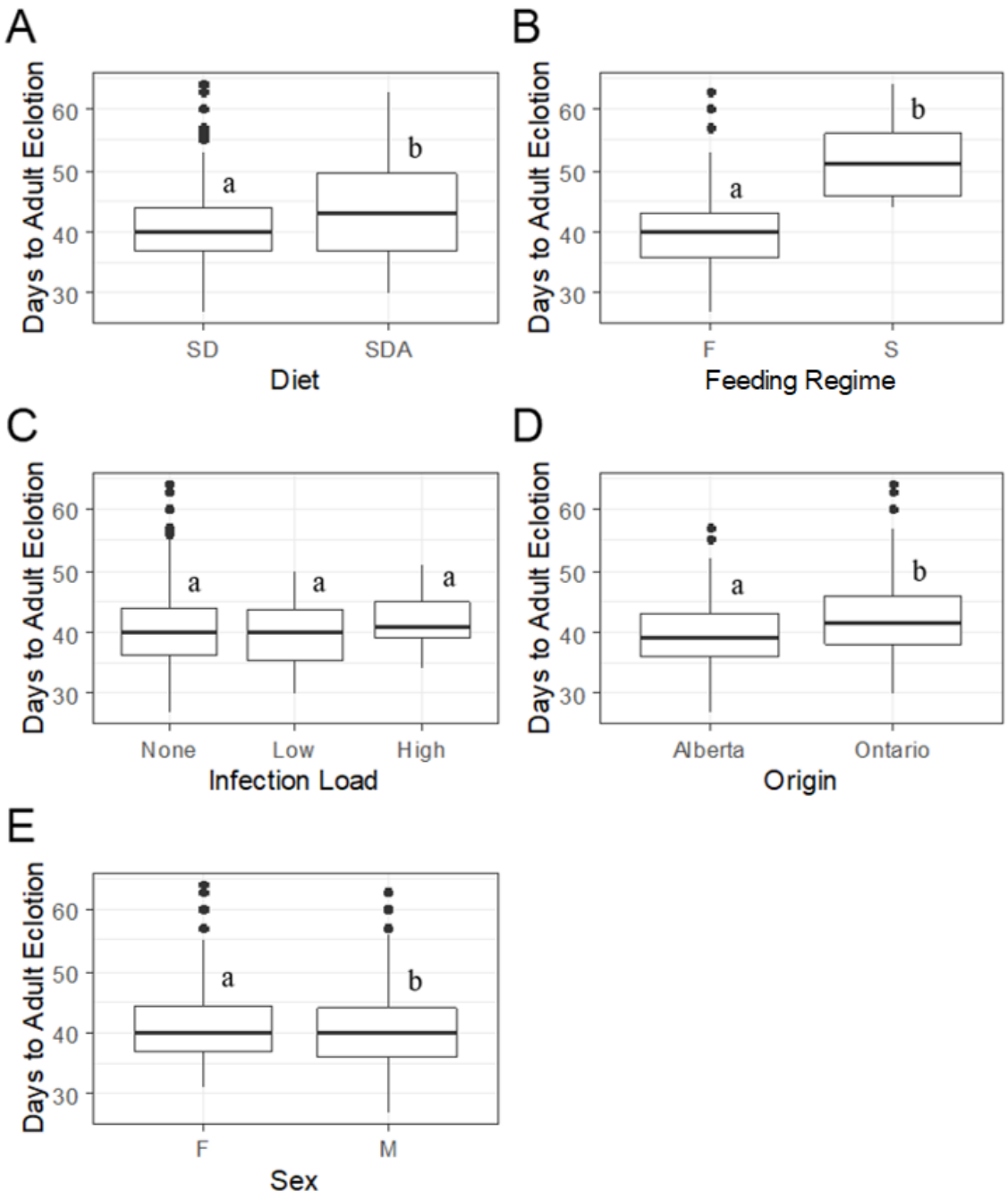

Figure 2-6. Boxplots of number of days required from egg hatch FTC to adult eclosion by (A) Diet: SD = Standard Diet, SDA = Standard Diet + trembling aspen foliage, (B) Feeding Regime: F = Full regime, S = Starved, (C) Infection Load: None = 0 spores, Low = 1 < spores < 100, High = 100+ spores per 10 fields of view at 400x magnification, (D) Origin: Alberta & Ontario, and (E) Sex: F = Females, M = Males. The midline of each box represents the median, whereas the bottom and top of each box indicates the first and third quartiles, respectively. The whiskers extending from each box indicate the maximum and minimum values and the black dots are outliers. Boxplots marked with different letters are significantly different (t test, $P < 0.05$).

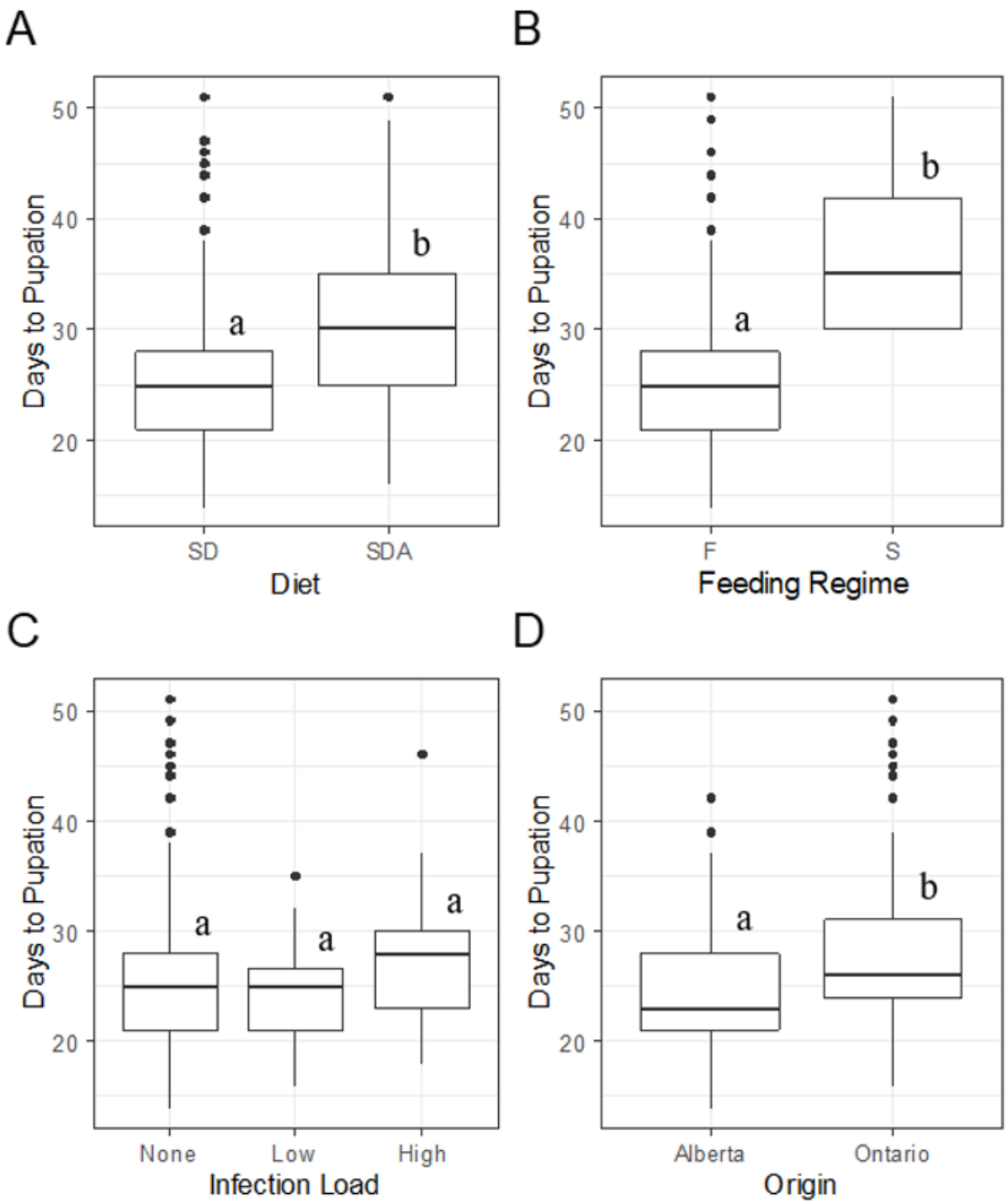

Figure 2-7. Boxplots of number of days required for development of FTC from egg hatch to pupation by (A) Diet: SD = Standard Diet, SDA = Standard Diet + trembling Aspen foliage, (B) Starvation: F = Full regime, S = Starved, (C) Infection Load: None = 0 spores, Low = 1 < spores < 100, High = 100+ spores per 10 fields of view at 400x magnification, and (D) Origin: Alberta & Ontario. The midline of each box represents the median, whereas the bottom and top of each box indicates the first and third quartiles, respectively. The whiskers extending from each box indicate the maximum and minimum values and the black dots are outliers. Boxplots marked with different letters are significantly different (t test, P < 0.05).

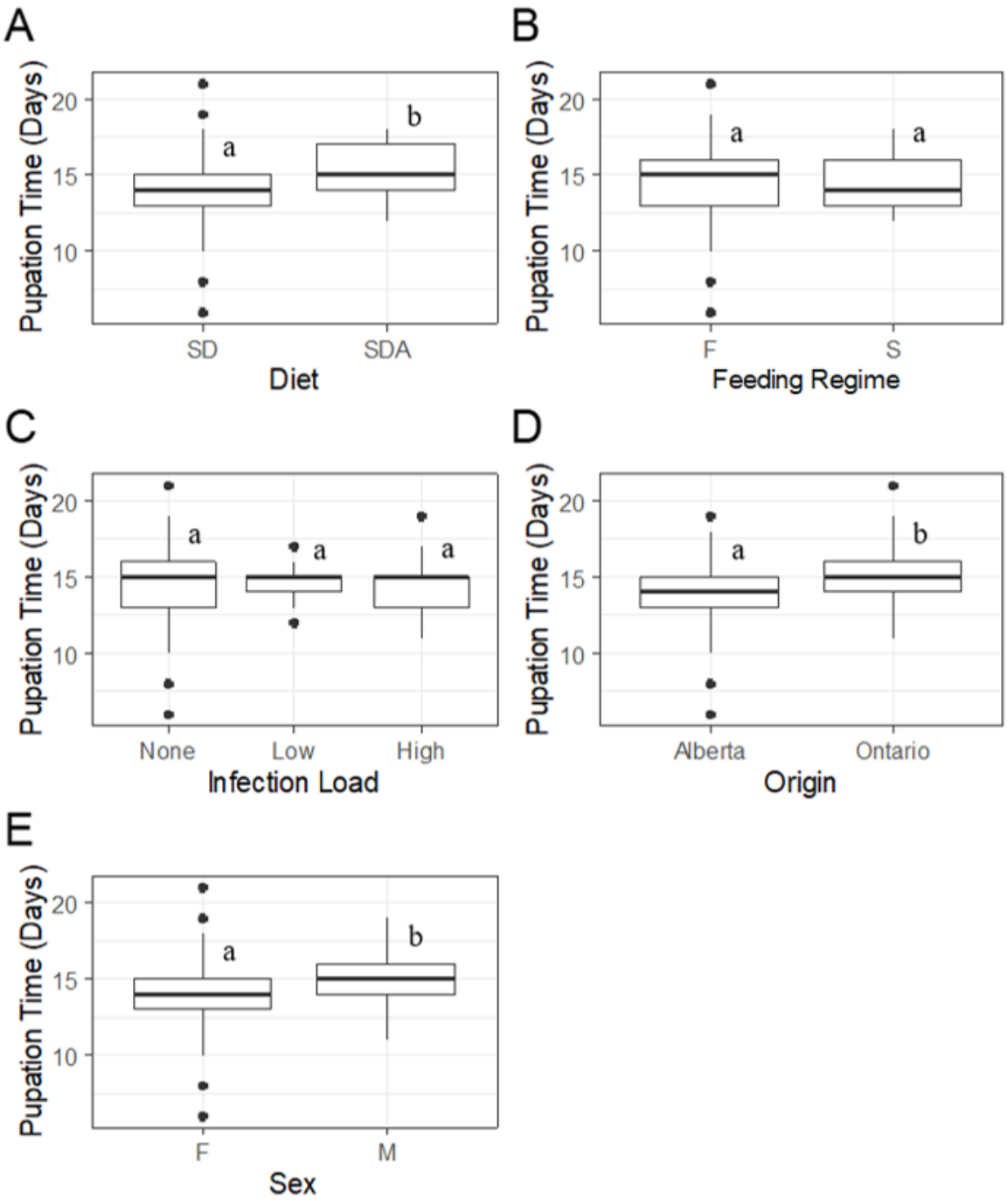

Figure 2-8. Boxplots of number of days required by FTC for pupal development by (A) Diet: SD = Standard Diet, SDA = Standard Diet + trembling aspen foliage, (B) Feeding Regime: F = Full regime, S = Starved, (C) Infection Load: None = 0 spores, Low = 1 < spores < 100, High = 100+ spores per 10 fields of view at 400x magnification, (D) Origin: Alberta & Ontario, and (E) Sex: F = Females, M = Males. The midline of each box represents the median, whereas the bottom and top of each box indicates the first and third quartiles, respectively. The whiskers extending from each box indicate the maximum and minimum values and the black dots are outliers. Boxplots marked with different letters are significantly different (t test, $P < 0.05$).

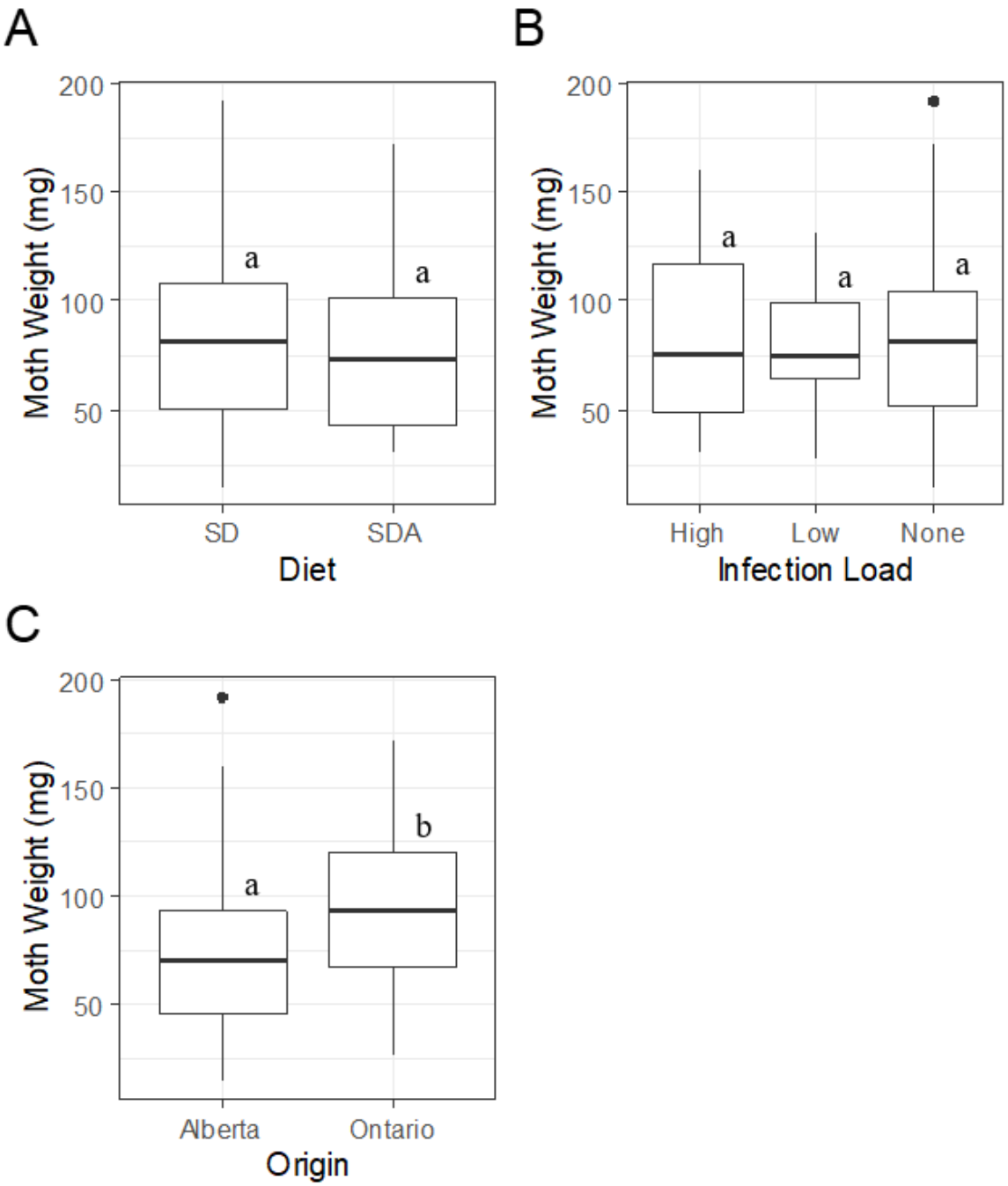

Figure 2-9. Boxplots of moth weight (mg) of female FTC by (A) Diet: SD = Standard Diet, SDA = Standard Diet + trembling aspen foliage, (B) Infection Load: None = 0 spores, Low = 1 < spores < 100, High = 100+ spores per 10 fields of view at 400x magnification, and (C) Origin: Alberta & Ontario. The midline of each box represents the median, whereas the bottom and top of each box indicates the first and third quartiles, respectively. The whiskers extending from each box indicate the maximum and minimum values and the black dots are outliers. Boxplots marked with different letters are significantly different (t test, $P < 0.05$).

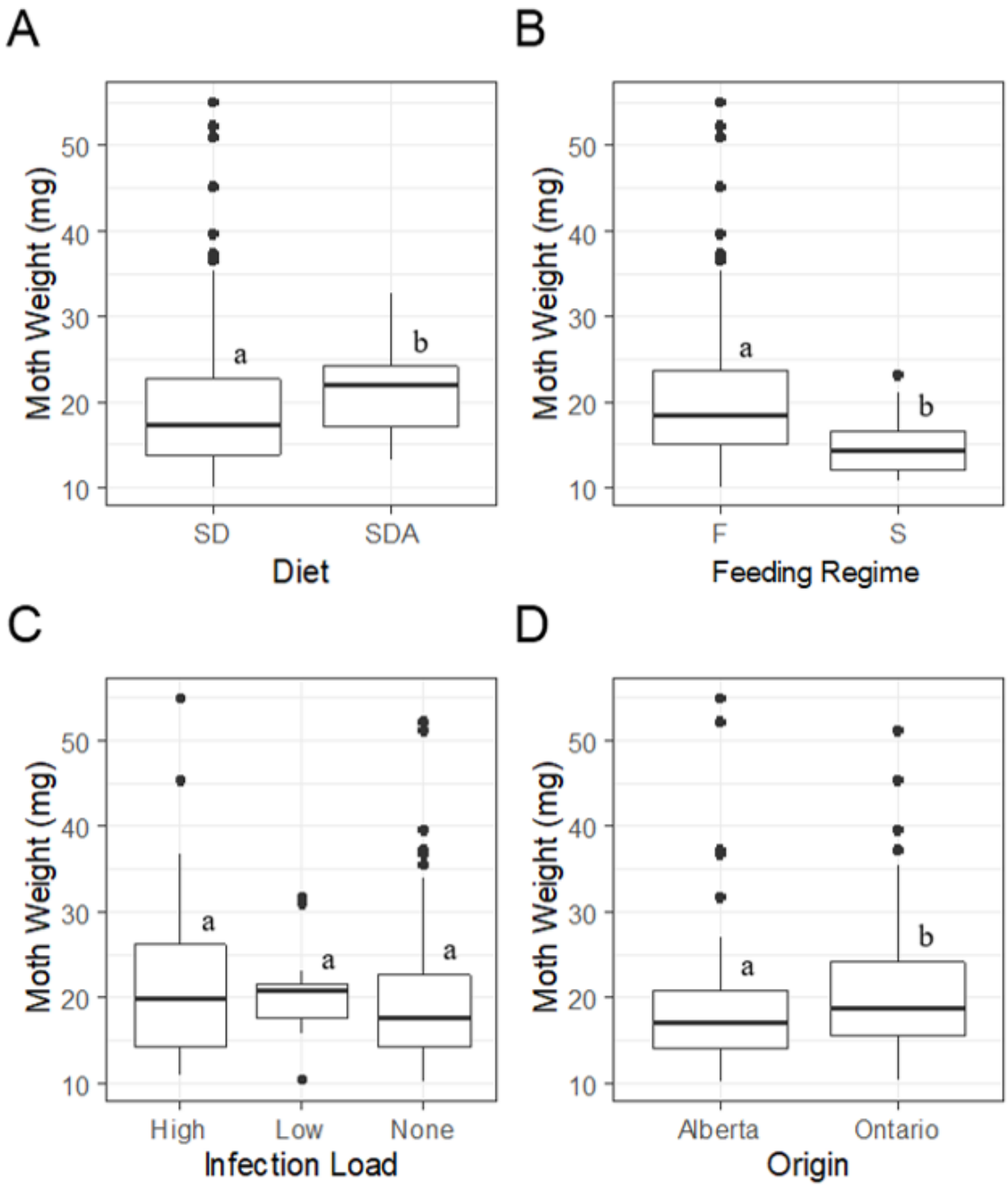

Figure 2-10.Boxplots of moth weight (mg) of male FTC by (A) Diet: SD = Standard Diet, SDA = Standard Diet + trembling Aspen foliage, (B) Feeding Regime: F = Full regime, S = Starved, (C) Infection Load: None = 0 spores, Low = 1 < spores < 100, High = 100+ spores per 10 fields of view at 400x magnification, and (D) Origin: Alberta & Ontario. The midline of each box represents the median, whereas the bottom and top of each box indicates the first and third quartiles, respectively. The whiskers extending from each box indicate the maximum and minimum values and the black dots are outliers. Boxplots marked with different letters are significantly different (t test, $P < 0.05$).

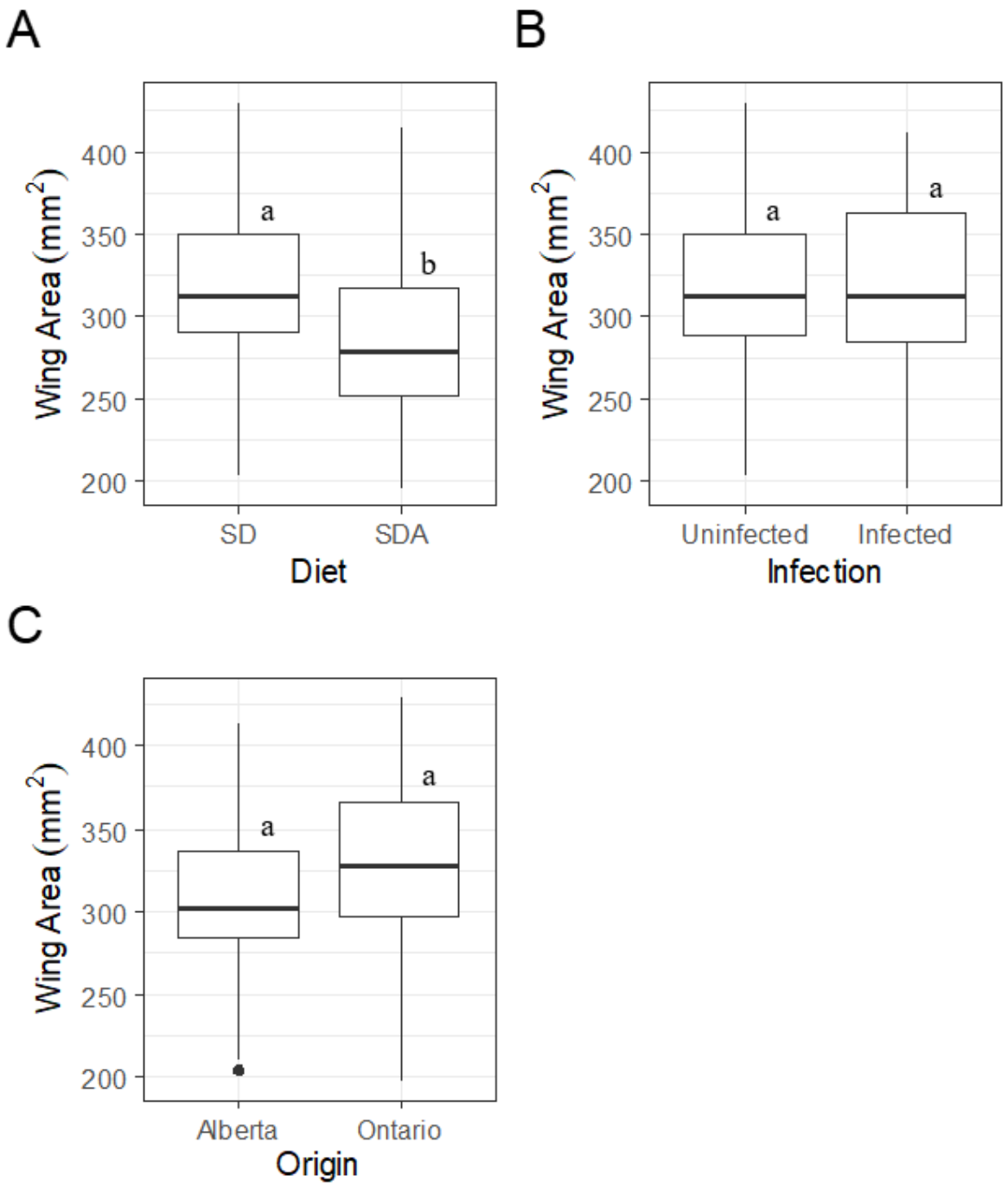

Figure 2-11. Boxplots of wing area (mm^2) of female FTC by (A) Diet: SD = Standard Diet, SDA = Standard Diet + trembling Aspen foliage, (B) Infection: Uninfected & Infected, and (C) Origin: Alberta & Ontario. The midline of each box represents the median, whereas the bottom and top of each box indicates the first and third quartiles, respectively. The whiskers extending from each box indicate the maximum and minimum values and the black dots are outliers. Boxplots marked with different letters are significantly different (t test, $P < 0.05$).

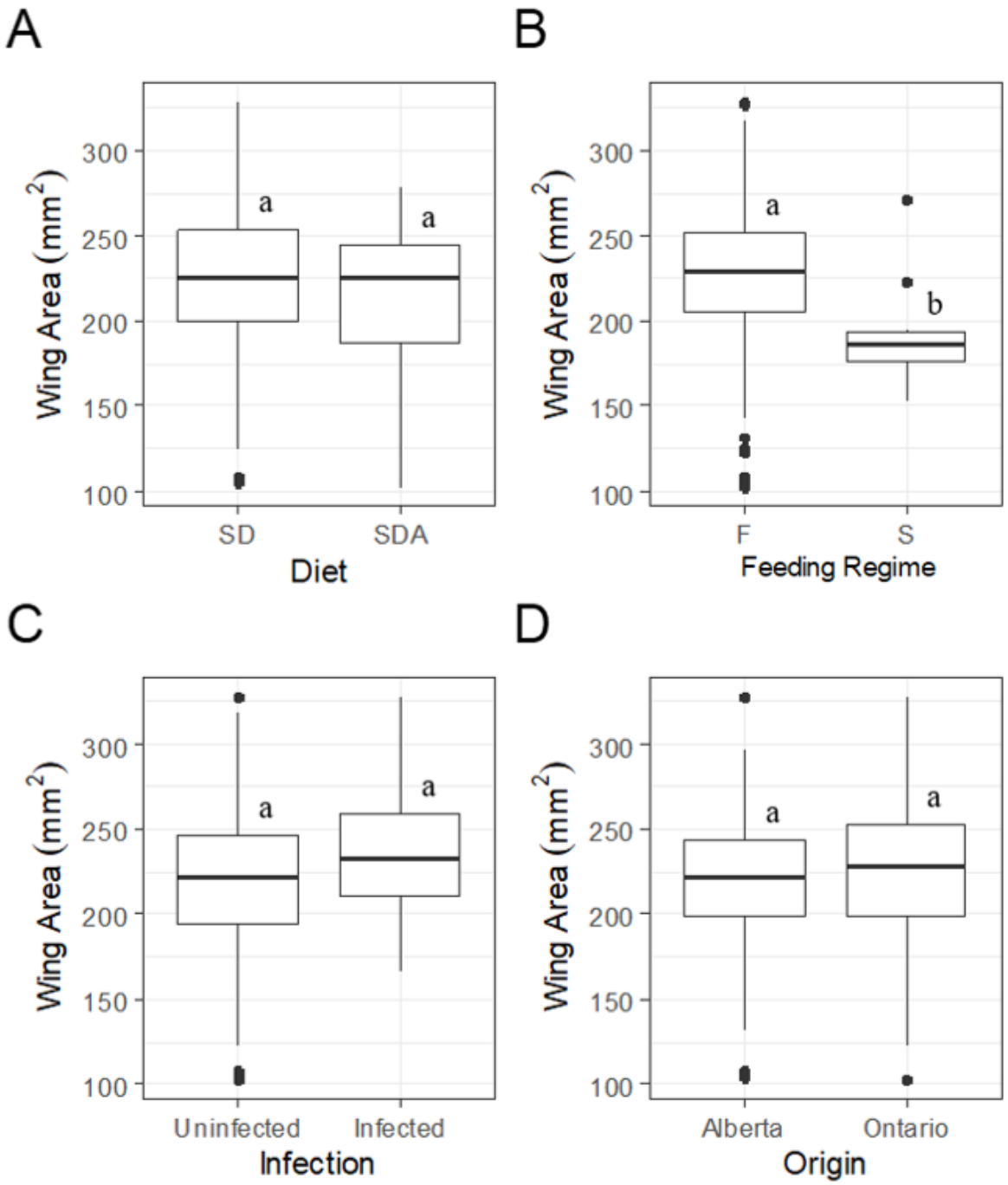

Figure 2-12. Boxplots of wing area (mm^2) of male FTC by (A) Diet: SD = Standard Diet, SDA = Standard Diet + trembling Aspen foliage, (B) Feeding Regime: F = Full regime, S = Starved, (C) Infection: Uninfected & Infected, and (D) Origin: Alberta & Ontario. The midline of each box represents the median, whereas the bottom and top of each box indicates the first and third quartiles, respectively. The whiskers extending from each box indicate the maximum and minimum values and the black dots are outliers. Boxplots marked with different letters are significantly different (t test, $P < 0.05$).

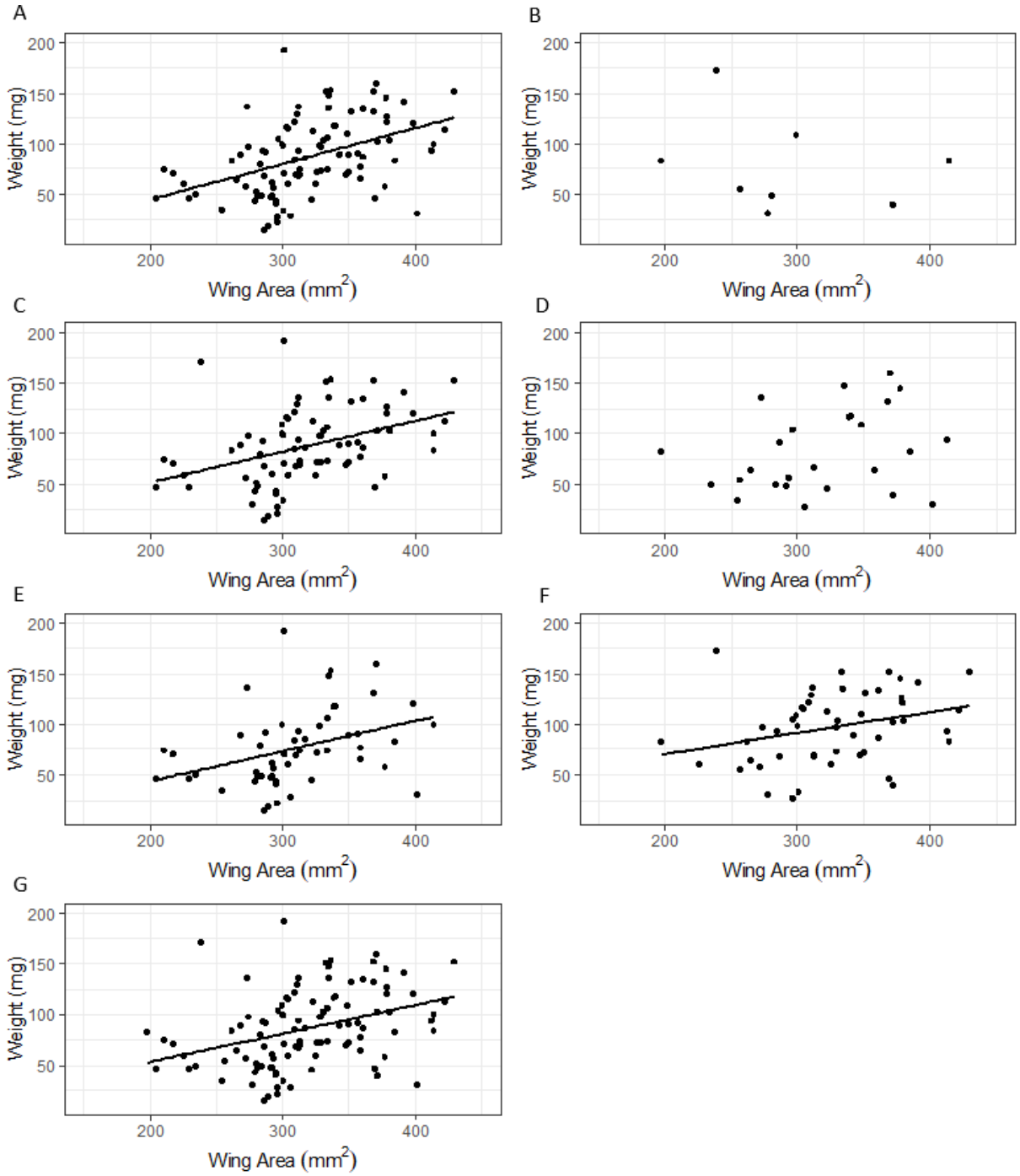

Figure 2-13. Allometric relationships between moth weight (mg) and wing area (mm^2) of female FTC moths by (A) Diet = SD, (B) Diet = SDA, (C) Infection = N, (D) Infection = Y, (E) Origin = Ontario, (F) Origin = Alberta, and (G) Feeding Regime = F.

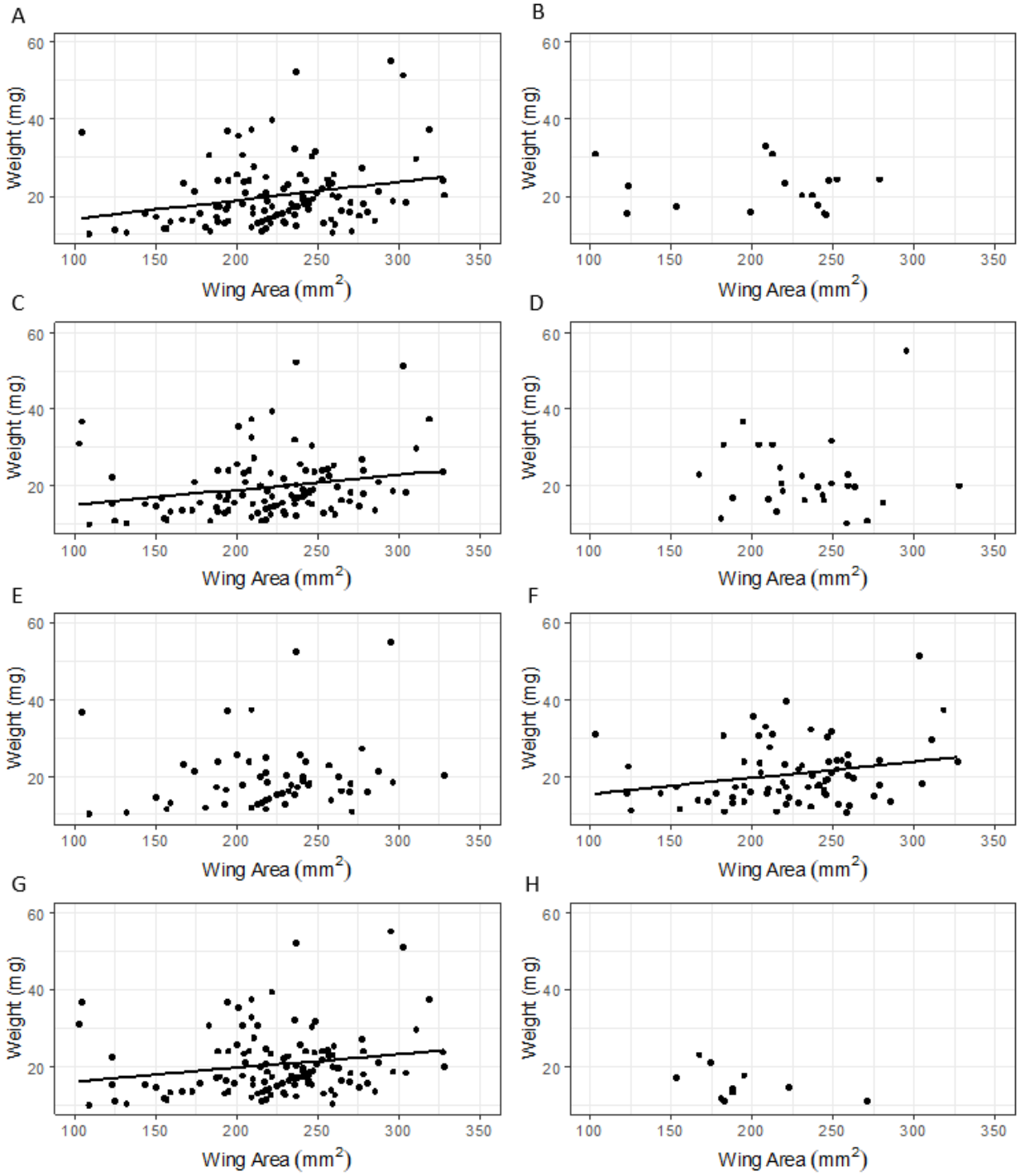

Figure 2-14. Allometric relationships between moth weight (mg) and wing area (mm^2) of male FTC moths by (A) Diet = SD, (B) Diet = SDA, (C) Infection = N, (D) Infection = Y, (E) Origin = Ontario, (F) Origin = Alberta, (G) Feeding Regime = F, and (H) Feeding Regime = S.

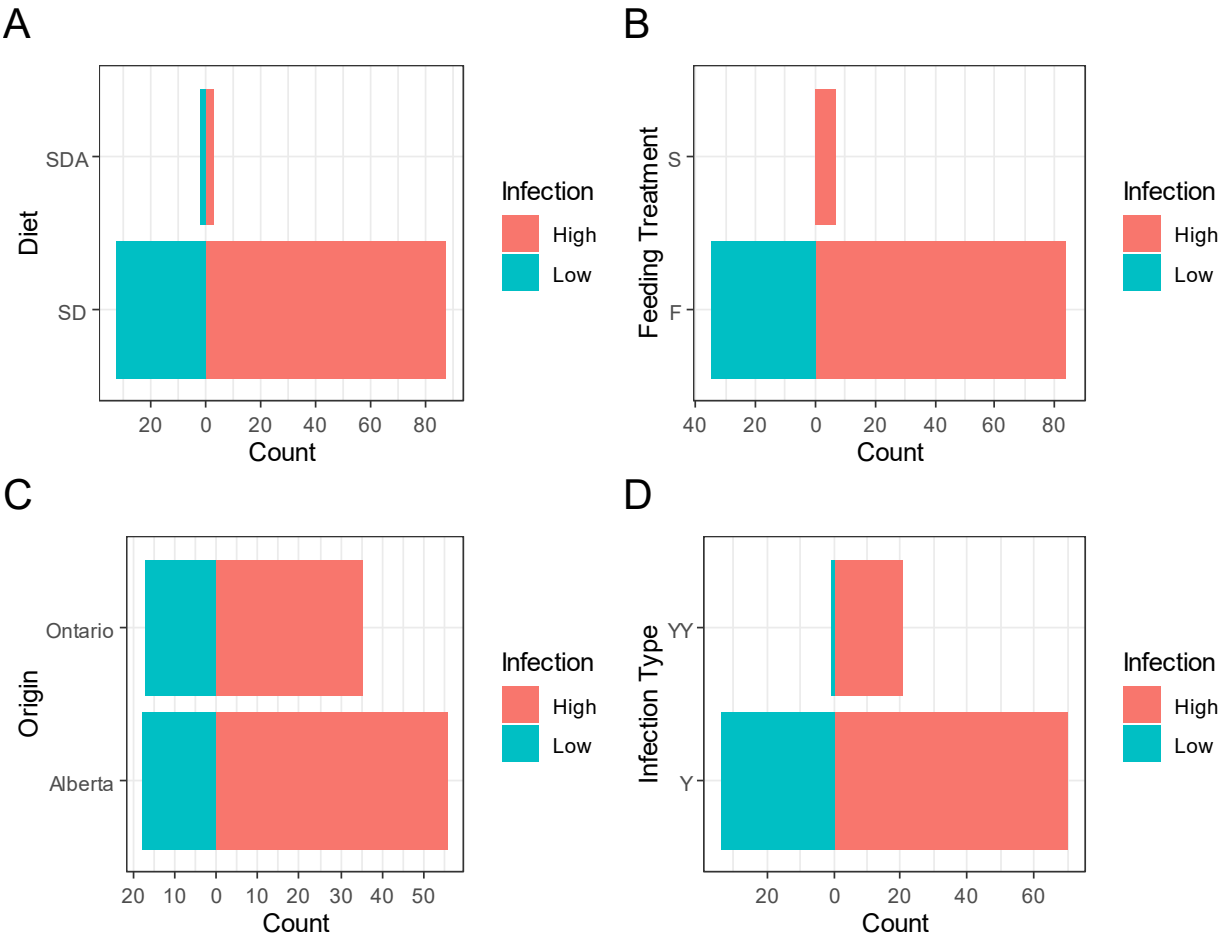

Figure 2-15. Pyramid plots of infection load (Low = 1 < spores < 100, High = 100+ spores per 10 fields of view at 400x magnification) in FTC by (A) Diet: SD = Standard Diet, SDA = Standard Diet + 1% lyophilized trembling aspen foliage, (B) Feeding Regime: F = Full regime, S = Starved, (C) Origin: Origin: Alberta & Ontario, and (D) Infection Status: Naturally infected and Inoculated at the 3rd larval instar.

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Chapter 3 – Effects of larval host and microsporidian infection on wing area of the forest tent caterpillar, *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae).

Abstract

Host affiliation and entomopathogenic infections play a major role in shaping population dynamics of the forest tent caterpillar (FTC), *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae). Both factors have been subject of extensive studies, but the interaction between plant phytochemicals and entomopathogenic infections has received limited attention. In two separate laboratory studies, we investigated the effects of larval diet and microsporidian infection on FTC wing area. Larvae were reared on trembling aspen, *Populus tremuloides* Michx (Malpighiales: Salicaceae), sugar maple, *Acer saccharum* Marshall (Sapindales: Sapindaceae), or an artificial standard diet. Natural levels of microsporidian infection were assessed through microscopy and categorized as none, low, or high depending on the number of spores counted. Wing area (mm^2) of adult moths was assessed as a measure of body size. High microsporidian infection was linked to reduced moth wing area, but there was no effect of infection on the probability of wing malformations. Further evidence is provided on the effects of larval host plant on FTC life history traits; FTC reared on fresh maple foliage were significantly smaller and had a higher probability of wing malformation than FTC reared on fresh aspen or artificial diet. No differences were observed in the severity of infection (spore load) as result of larval diet. Forest tent caterpillar from Ontario displayed significantly higher spore concentration than insects from Alberta, suggesting populations from Alberta and Ontario may be at different stages in the population cycle.

Introduction

It is now established that population dynamics of phytophagous insects are affected by the quality of the host (Boggs, 1992; Roff, 2009; Scriber & Slansky, 1981). Plant host affiliation is dictated by

differences in nutritional value and morphological and chemical defenses of plants, which can alter the fitness of herbivorous insects (Bernays & Chapman, 2007; Schoonhoven et al., 2005), as well as their susceptibility to entomopathogens (Cory & Hoover, 2006). For example, the corn earworm, *Helicoverpa zea* Boddie (Lepioptera: Noctuidae), displays greater resistance to the nucleopolyhedrovirus (NPV) *Baculovirus heliothis* (Caudovirales: Baculoviridae) when reared on upland cotton, *Gossypium hirsutum* Linnaeus (Malvales: Malvaceae), compared to tomato, *Solanum lycopersicum* Linnaeus (Solanales: Solanaceae), or artificial diet (Forschler et al., 1992). Here, we examine the potential contributions of host affiliation (bottom-up), entomopathogenic infections (top-down) and their interaction on life history traits of an outbreaking forest lepidopteran, the forest tent caterpillar, *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae).

The forest tent caterpillar (FTC) is a gregarious lepidopteran insect native to North America, and a major defoliator of deciduous hardwood trees (Fitzgerald, 1995). The forest tent caterpillar is phenologically synchronized with its preferred hosts (Gray & Ostaff, 2012) and it obtains all its nutrients through larval feeding, as the adults are short lived and lack functional mouthparts (Fitzgerald, 1995). Dietary effects of different larval hosts translate into adult life history traits (Boggs, 1992). Forest tent caterpillars that feed on trembling aspen, *Populus tremuloides* Michx (Malpighiales: Salicaceae), as larvae have higher fecundity and larger eggs than FTC reared on sugar maple, *Acer saccharum* Marshall (Sapindales: Sapindaceae) (Trudeau et al., 2010). This may be due to lower sugar and higher secondary metabolite (phenolic compounds and tannins) concentrations in sugar maple, as compared to trembling aspen (Lorenzetti, 1993). Host phytochemistry is also linked to changes in population and outbreak dynamics in FTC (Donaldson & Lindroth, 2008). For example, in Québec, FTC outbreaks occur more frequently in trembling aspen compared to sugar maple forests (Cooke & Lorenzetti, 2006).

Larval diet can also affect the trade-off between reproduction and dispersal in FTC moths. Larvae fed artificial diet *ad libitum* have higher wing loading compared to foliage-fed FTC (Evenden et al.

2015a). Moreover, the protein-carbohydrate ratios in artificial diets alter resource allocation to reproduction (Colasurdo et al., 2009). Larvae reared on high carbohydrate diets allocate more resources to somatic tissues compared to reproductive tissues (ovaries), whereas larvae fed diets with equal or higher protein-to-carbohydrate content allocate resources to reproductive tissues and egg development. The allometric relationship between wing area and body weight in FTC is stronger for larvae that feed on fresh aspen foliage than on artificial diet (Evenden et al. 2015a), but the addition of 1% lyophilized trembling aspen to artificial diet resulted in a loss of this allometric relationship (Chapter 2). It is clear that host affiliation plays a major role in dictating population dynamics of FTC, however, other factors, such as climate, population density, and natural enemies may also play a role and interact with larval diet to impact population cycling.

Microsporidia are unicellular eukaryotic parasites that cause chronic disease in insects (Becnel & Andreadis, 1999). Forest tent caterpillars are often naturally infected with the microsporidian pathogen *Nosema disstriae* (Dissociodihaplophasida: Nosematidae) (Thomson, 1959), however, a few other species in the genera *Pleistophora* (Wilson, 1977b), *Vairimorpha* (Wilson, 1984), and *Thelohania* (Smirnoff, 1968) cause disease in FTC. Severe microsporidian infection in FTC causes larval mortality (Wilson, 1977b; Wilson, 1984), whereas sublethal infection is associated with delayed development time, reduced weight, heavy spore concentration in the silk glands, and changes in the allometric relationship between adult weight and wing area (Chapter 2; Wilson, 1984). High microsporidian infection rates of FTC occur in established populations at high densities (Fitzgerald 1995), but not necessarily in new populations at high densities (Jones & Evenden 2008). This suggests that microsporidia may influence population dynamics of FTC.

To our knowledge, only one study has explored the potential interaction between larval diet and microsporidian infection in FTC (Chapter 2). This study utilized qualitative and quantitative manipulations of artificial diets and did not assess possible interactions with fresh foliage from host

trees. The study found no significant alteration of FTC susceptibility to *N. disstria* infection under restricted nutritional availability or modified larval diets. There is, however, an interaction between larval diet and disease expression caused by *Bacillus thuringiensis* var *Kurstaki* (Bacillales: Bacillaceae) (*Btk*) in FTC. Larval mortality from *Btk* is greater when larvae are fed sugar maple compared to trembling aspen (Kouassi et al., 2001). This suggests that the phytochemistry of a secondary host (sugar maple) contributes to the pathogenicity of *Btk*. Tritrophic interactions among entomopathogens, FTC, and their larval host, may depend on the type and level of entomopathogen infection. The current experiment assesses the interaction between larval diet and microsporidian infection in FTC when reared on fresh trembling aspen, sugar maple foliage, or artificial diet.

Materials and Methods

Two experiments were conducted to test the hypothesis that sublethal microsporidian infection of FTC larvae interacts with larval diet to influence moth size, as measured by wing area. Experiments were conducted in the Evenden (2014) and Flaherty (2018) labs using insects sourced in both Alberta and Ontario.

Caterpillar rearing

2014 experiment

Egg masses from both Ontario and Alberta were used in the experiment. Ontario egg masses were collected in the winter of 2013-14 by the Great Lakes Forestry Centre, ON from two separate FTC populations, one near Dryden, ON (49.7801°N, 92.8370°W) and one near Kenora, ON (49.7670°N, 94.4894°W). Alberta egg masses were collected near Peace River (N 57° 20.503'W 117° 31.921'). Egg masses were stored in paper bags at 4°C until use. All egg masses were washed in a 5% sodium hypochlorite solution for 2 minutes, rinsed under water for 5 minutes, and washed again in a 0.05%

bleach solution. On 20 May 2014, egg masses were placed individually in Petri dishes (100 x 15mm) and held under ambient light and temperature (~22°C). Upon hatching, 20 egg masses from the Dryden and Alberta populations and 10 egg masses from the Kenora population were selected for testing. To control for larval genetics, half of the larvae from each egg mass was assigned to diet treatments on 23 May, 2014 of either fresh aspen foliage, or artificial diet (Addy 1969). Fresh aspen foliage was obtained as needed from trees at several sites in south Edmonton throughout the experiment. Foliage was used within 3 days of collection and was held at 4°C until use. Before feeding, leaves were sterilized by soaking branches in a 0.06% sodium hypochlorite solution for 5 minutes and rinsed in water. The branches were then air dried and placed under a UV light for 10 minutes on each side. Larvae were reared in Petri dishes (100 x 15mm) and food was provided *ad libitum*. Old food was removed at each feeding. To accommodate feeding of late larval instars, larvae were moved on 2 June, 2014 to larger transparent containers (4-6 L). Upon pupation, pupae were transferred to individual containers (100 ml) to allow moth eclosion. Moths were separated by sex and frozen prior to dissection to determine microsporidian spore load and wing area measurements (see below).

2018 experiment

Forest tent caterpillar egg masses were collected from trembling aspen stands near Sault Ste. Marie, ON (46.5136°N, 84.3358°W) on 2-3 May, 2018 and shipped to Edmonton, AB in a refrigerated container. Egg masses hatched during the journey, so it was impossible to distinguish and isolate related individuals. On 10 May 2018, FTC larvae were haphazardly assigned to replicates within diet treatments. Twenty-two replicates of 20 larvae were reared on fresh aspen foliage, 9 replicates of 20 larvae on fresh sugar maple foliage, and 20 replicates of 30-50 larvae on a modified Addy artificial diet, which were reduced to 20 individuals per replicate on 15 May 2018 (Addy 1969, Appendix C). For foliar diets, fresh aspen was collected at different locations in central Alberta to ensure genetic diversity, and fresh maple was collected from a single tree at 10958 85 Ave, Edmonton, AB. The lack of genetic diversity in maple

foliage was due to the lack of available trees in the area. Fresh foliage was collected using pole pruners and stored in coolers containing water saturated floral foam blocks and ice packs until transport to the laboratory. Leaves were sterilized by soaking branches in a 0.06% sodium hypochlorite solution for 5 minutes and rinsed in water. The branches were then air dried and placed under a UV light for 10 minutes on each side. Larvae in artificial diet treatments were reared in 177 ml plastic cups containing approximately 85 ml of diet. Larvae from the aspen and maple foliage diet treatments were reared in 473 ml containers, because extra space was needed to house floral picks in which foliage were placed. Diets were provided *ad libitum*; fresh foliage was replaced every 2-3 days, and artificial diet was replaced every 5-7 days. Caterpillars were reared under laboratory conditions in a Caron growth chamber at 22°C, 55% humidity, 16L:8D photoperiod. Dead larvae and frass were removed weekly, and containers were wiped down with 70% ethanol. Upon pupation, pupae were transferred to individual cups to allow moth eclosion. Moths were separated by sex, checked for microsporidian infection status and load, and dissected for wing area measurements (see below).

Wing Area Measurements

In the 2014 experiment, the right forewing and the right hindwing of each moth were carefully removed and glued onto a white piece of paper. The paper was covered by a clear plastic sheet cover and scanned at 118.1 pixels/cm (300 PPI) in JPEG format. ImageJ software (1.34s United States National Institute of Health) was used to process the wings into a binary black and white image to determine wing area in mm^2. To obtain total wing area, the two wing measurements were added together. In the 2018 experiment, both pairs of wings from each moth were assessed. To compare the results with data analyzed from 2014, the total wing area from the 2018 experiment was divided by two.

Microsporidia Infection Assessment

Forest tent caterpillar adults were assessed for microsporidian infection status and infection load. The abdomen of adult moths was removed and crushed with a pestle in a microcentrifuge tube (1.5ml) containing 500 µl of dH_2O. The solution was vortexed for 5 seconds and 25 µl of liquid was pipetted onto a microscope slide. Ten fields of view at 400x were examined for the presence of microsporidian infection. The moths were assessed as infected (Y) or uninfected (N) based on the presence or absence of microsporidia spores. The total number of spores in the examined area was counted to assess "infection load" (0 = None, 1-100 = Low, 100+ = High).

Statistical Analyses 2014 Experiment

Not all moths were assessed for microsporidian infection, therefore, the entire dataset (n = 860) could only be used to explore the effects of diet, origin, and their interaction on wing area. Three outliers were removed from the dataset to accommodate model fit. A subset of the moths for which microsporidian infection was determined (n = 495) was used to assess the impact of sex, diet, and origin on microsporidian infection load (spore nuclear division within the host), as well as the effect of microsporidian infection and its interaction with diet and origin on total wing area. Wing measurements in 2014 were only conducted on those individuals with full wing development. Models included egg mass nested within origin as random effect. All analyses were conducted in R Studio version 1.0.136 (2018) with a significance level of 0.05 (Table 3-1).

Wing Area - Diet and Origin

Given the morphological difference between male and female FTC moths, each sex was analyzed separately. Two Linear Mixed-Effect Models (LMMs) were used to explore the effects of Diet, Origin, and their interaction on the total wing area. Residual normality and homoscedasticity were met. Type III Analysis of Variance with Satterthwaite's approximation was used to detect significance for both

models, as well as to estimate F-values and residual degrees of freedom. Given the interaction between Diet and Origin in both models, a pair-wise comparison was computed using the emmeans package (Lenth, 2020) with a Kenward-Roger approximation method for degrees of freedom and a Tukey p-value adjustment.

Wing Area - Infection

Two Linear Mixed-Effect Models (LMMs) were used to explore the effects of infection and its interaction with Diet and Origin on total wing area of male and female FTC moths. Residual normality and homoscedasticity were met. Not all moths were assessed for microsporidian infection; only the insects that were graded for infection (n = 211 females, n = 284 males) were used in the analyses. Subsequent models had infection load (None: 0 spores count, Low: 1-100 spores count, High: 101+ spores count) as an independent variable instead of infection incidence (Y/N) to understand the effects of spore concentration on wing area. Non-significant interaction effects were removed from models using ANOVA hypothesis testing. Type II Analysis of Variance with Satterthwaite's approximation was used to detect significance, as well as to estimate F-values and residuals degree of freedom. Estimated Marginal Means (EMMs) with Tukey method p-value adjustments were used to identify differences in wing area by infection load in the second series of models.

Infection

Infected moths were separated by infection level as low (1-100 spores) and high (> 100 spores) to determine the effects of Diet, Sex, Origin, and their interaction on infection load in adult FTC. A generalized linear mixed-effects model (GLMM) for binary outcomes was used. Overdispersion, Pearson χ^2 Goodness of Fit, and Likelihood Ratio tests indicated the model fit the data. A type II Wald χ^2 test was used to detect significance and Estimated Marginal Means (EMMs) with a Tukey method p-value adjustment were compared to identify significant differences.

Statistical Analysis 2018 Experiment

In the experiment conducted in 2018, several individuals emerged with malformed wings that presented as outliers in the data frame. Frequency curves of the dataset, alongside a visual inspection of the specimens, were used to identify malformed wings (females < 100mm^2, males < 70mm^2, Appendix F). Individuals with malformed wings were removed from the dataset and analyzed separately. All models included repeat number (individual container) as random effect. All analyses were conducted in R Studio version 1.0.136 (2018) with a significance level of 0.05 (Table 3-3).

Wing Area

Forest tent caterpillar moth wing area was analyzed separately for males and females. Only individuals with normal wings (females ≥ 100mm^2, males ≥ 70mm^2) were included in the models. Two Linear Mixed-Effects Models (LMMs) were used to explore the effects of Diet, Infection, and their interaction on the total wing area. Residual normality and homoscedasticity were met. No significant interactions were found, and the models were simplified by ANOVA hypothesis testing. Type II Analysis of Variance with Satterthwaite's approximation was used to detect significance, as well as to estimate F-values and residuals degree of freedom. Estimated Marginal Means (EMMs) with a Tukey method p-value adjustment were compared to identify significant differences among diet treatments. Subsequent models had infection load (None: 0 spores count, Low: 1-100 spores count, High: 101+ spores count) as an independent variable instead of infection incidence (Y/N) to understand the effects of spore concentration on wing area. The models passed validation tests, and their residuals were normally distributed. Type II Analysis of Variance with Satterthwaite's approximation was used to detect significance, as well as to estimate F-values and residual degrees of freedom.

Malformed Wings

The prevalence of malformed wings in moths in the 2018 data set was analyzed using a generalized linear mixed-effects model (GLMM) for binary outcomes. The presence of malformed wings

(Y/N) was the dependent variable and Diet, Sex, Infection, and their interactions were the independent variables. Overdispersion, Pearson χ^2 Goodness of Fit, and Likelihood Ratio tests indicated the model satisfied the data. A type II Wald χ^2 test was used to detect significance. Subsequent models had infection load (None: 0 spores count, Low: 1-100 spores count, High: 101+ spores count) as an independent variable instead of infection incidence (Y/N) to understand the effects of spore concentration on wing malformation. Overdispersion, Pearson χ^2 Goodness of Fit, and Likelihood Ratio tests indicated the model fit the data. A type II Wald χ^2 test was used to detect significance. Estimated Marginal Means (EMMs) with a Tukey method p-value adjustment were compared to identify significant differences among diet treatments.

Infection Load

Infected moths were separated by infection level as low (1-100 spores) and high (> 100 spores). To determine the effects of Diet, Sex, and their interaction on infection load in adult FTC, a generalized linear mixed-effects model (GLMM) for binary outcomes was used. Overdispersion, Pearson χ^2 Goodness of Fit, and Likelihood Ratio tests indicated the model satisfied the data. A type II Wald χ^2 test was used to detect significance.

Results

Wing Area (2014)

Diet and Origin

The interaction between Diet and Origin affected wing area in both male ($F_{2,467.6} = 5.552$, $p = 0.004$) and female ($F_{2,357.4} = 4.601$, $p = 0.011$) moths. Male moths from AB that were reared on artificial diet developed significantly larger wings ($t_{481} = 3.881$, $p = 0.002$) than aspen-fed male moths from AB. Comparable significant results are seen in male moths from Dryden, Ontario ($t_{383} = 3.864$, $p = 0.002$), but

not for male moths from Kenora, Ontario (t_{484} = 0.812, p = 0.965) (Figure 3-1). Similarly, female moths from AB that were fed artificial diet developed significantly larger wings (t_{363}= 3.418, p = 0.009) than aspen-fed female moths from AB. Comparable significant results occur in female moths from Dryden, Ontario (t_{304}= 5.035, p < 0.001), but not in female moths from Kenora, Ontario (t_{357} = 0.516, p = 0.996) (Figure 3-2).

Infection

Infected individuals had smaller wing area in both male ($F_{1,203.6}$ = 12.270, p < 0.001) and female ($F_{1,210.8}$ = 7.096, p = 0.008) moths than uninfected moths (Figure 3-3). Wing area was significantly affected by microsporidian infection load in both male ($F_{2,225.8}$ = 6.177, p = 0.002) and female ($F_{2,199.4}$ = 6.891, p = 0.001) moths (Figure 3-4). Uninfected male moths had significantly larger wings than infected males at low (t_{281} = 3.230, p = 0.004) and high (t_{250} = 2.559, p = 0.030) levels of infection. No statistical difference in wing area was detected between male moths with low or high infection levels (t_{283} = -0.341, p = 0.938). Uninfected female moths had wings that were significantly bigger than those of females with high spore loads (t_{206} = 3.596, p = 0.001), but not low spore loads (t_{191} = 1.130, p = 0.497) females. Furthermore, wings from female moths with low infection were statistically bigger than those of females with high infection (t_{211} = 2.469, p = 0.038).

<u>Infection (2014)</u>

The spore load of FTC moths was affected by both Sex (χ^2 = 9.071, p = 0.003) and Origin (χ^2 = 13.976, p = 0.001), but not Diet (χ^2 = 0.317, p = 0.573) (Figure 3-5). Females were statistically more likely than males to be heavily infected (χ^2 = 9.071, p = 0.003). Furthermore, Alberta moths had a lower spore load than FTC from either site in Ontario, Dryden (Z = -3.734, p = 0.001) and Kenora (Z = -3.063, p = 0.006). There was no statistical difference, however, in microsporidia spore concentration between moths from the two sites in Ontario (Z = 0.462, p = 0.832).

Wing Area (2018)

In the 2018 experiment, microsporidian infection did not affect the wing size of male ($F_{1,48.3}$ = 0.149, p = 0.701) or female ($F_{1,160}$ = 0.474, p = 0.492) FTC moths (Figure 3-6). There was, however, a statistical effect of larval diet on wing area for both male ($F_{2,34.7}$ = 78.078, p < 0.001) and female ($F_{2,160}$ = 40.098, p < 0.001) moths (Figure 3-7). Wings of FTC adults that were reared on fresh maple foliage were significantly smaller than male ($t_{40.5}$ = -4.960, p < 0.001) and female ($t_{53.7}$ = -6.085, p < 0.001) moths reared on the artificial diet, and males ($t_{34.2}$ = -11.883, p < 0.001) and females ($t_{54.3}$ = -8.834, p < 0.001) moths reared on fresh aspen. Furthermore, moths reared on fresh aspen developed the largest wing area in both males and females, which were statistically larger than the wings of male ($t_{37.7}$ = 7.935, p < 0.001) and female ($t_{31.4}$ = 4.655, p < 0.001) moths reared on the artificial diet. There was no statistical difference of microsporidian infection load (None, Low, High) on wing area in either male ($F_{2,79.1}$ = 0.154, p = 0.856) or female ($F_{2,159}$ = 0.221, p = 0.802) moths (Figure 3-8).

Malformed Wings (2018)

Females of FTC were statistically more likely (χ^2 = 7.152, p = 0.007) to develop malformed wings than males, regardless of infection or diet (Figure 3-9). Males had malformed wings in 14.1% of cases (n = 29, N = 205), while 23.4% of females had malformed wings (n = 50, N = 214). Wing malformation was affected by larval diet (χ^2 = 8.158, p = 0.017). Larvae reared on fresh maple (FM) developed into moths with malformed wings in 30.9% of cases (n = 25, N = 81), compared to only 13.8% of moths that developed from larvae reared on fresh aspen (FA, Z = 2.785, p = 0.015, n = 21, N = 152). Larvae reared on the artificial diet developed into moths with malformed wings in 17.7% of individuals (n = 33, N = 186) and did not differ statistically from larvae reared on maple (Z = -2.167, p = 0.077) or aspen (Z = 0.831, p = 0.684).

Although no statistical difference (Z = -2.330, p = 0.182) was found in the prevalence of malformed wings between uninfected and infected moths fed on fresh maple, a biological trend is visible. Infected moths reared on fresh maple produced malformed wings in 40.8% of cases (n = 20, N = 49), while uninfected moths produced malformed wings in 15.6% of cases (n = 5, N = 32). No statistical difference in the incidence of wing malformation was found between infected and uninfected individuals fed on fresh aspen or the synthetic diet.

Infection (2018)

Sex (χ^2 = 0.580, p = 0.446) and Diet (χ^2 = 3.019, p = 0.221) did not affect the spore load in FTC moths (Figure 3-10). No significant difference in infection load was detected among larvae fed the different diets, but a clear trend is visible. Twenty six percent of the FTC fed on the artificial diet had a high spore load (n = 18, N = 69), compared to 42.2% (n = 35, N = 83) for those fed fresh aspen and 40.8% (n = 20, N = 49) for those fed fresh maple.

Discussion

Experiments comparing diets of fresh foliage to artificial diet in both 2014 and 2018 illustrate that moth size was not affected by an interaction between diet and microsporidian infection. These findings coincide with previous work on FTC that illustrates separate effects but no interaction effect of diet and microsporidian infection on adult life history traits in this species (Chapter 2). Similarly, there was no interaction between diet quality and infection with *N. fumiferanae* on spruce budworm pupal mass, although diet and infection factors alone influenced life history traits (van Frankenhuyzen & Li, 2016). In contrast, supplementation of the allelochemical xanthotoxin to larval diet significantly delayed mortality by microsporidian infection in the cabbage looper, *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae) (Carloye et al., 1998). Moreover, Smirnoff (1967) showed that several plants extract

significantly reduce susceptibility of the ugly-nest caterpillar, *Archips cerasivorana* Fitch (Lepidoptera: Tortricidae), to microsporidian infection (*Nosema* genus). Among the tested compounds, onion extract was especially successful at inhibiting infection (Smirnoff, 1967), however, the same extract had no impact on the susceptibility of the cabbage looper to infection with *Vairimorpha* sp. (Carloye et al., 1998). These studies suggest that some secondary chemicals may present antiparasitic activity against microsporidia, but the results are equivocal, and the mechanisms are still largely unknown. Future studies should determine if there is an interaction between larval diet and microsporidian infection that influences mortality of FTC.

In our study, FTC wing area was affected by infection status in the 2014 but not the 2018 experiment. Uninfected male and female moths had wings that were significantly larger than heavily infected moths in 2014. Wing area of lightly infected moths was intermediate between uninfected and heavily infected moths. The probability of wing malformation, however, was not affected by microsporidian infection, confirming previous findings (Chapter 2). Microsporidian infection causes reduced wing size and/or wing malformations in other adult insects. The desert locust, *Schistocerca gregaria* Forsskål (Orthoptera: Acrididae), displays reduced wing size when infected with spores of *Paranosema (Nosema) locustae* (Microsporidia: Nosematidae) (Tounou, 2007). Increased prevalence of wing malformations is observed when the melanopline grasshopper, *Dichroplus maculipennis* Blanchard (Orthoptera: Acrididae) is infected with *P. locustae* (Mariottini & Lange, 2014). Fujii & Tamashiro (1972) reported alterations in wing holding position and damaged muscle tissues in the Oriental Fruit Fly, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae), when infected with the microsporidian *N. tephrititae* (Microsporidia: Nosematidae). Interestingly, even the parasitoid wasp *Pediobius foveolatus* Crawford (Hymenoptera: Eulophidae) presents an increased probability of wing malformation when parasitizing Mexican bean beetles, *Epilachna varivestis* (Coleoptera: Coccinellidae), infected with spores of *N. epilachnae* (Microsporidia: Nosematidae) (Own & Brooks, 1986).

In the Lepidoptera, the effect of microsporidian infection on wing area is inconsistent. Wing area of the moth *Ancylis sativa* Liu (Lepidoptera: Tortricidae) is significantly reduced with increasing microsporidian spore load (Zhang et al., 2012). On the contrary, wing area of the large aspen tortrix, *Choristoneura conflictana* Walker (Lepidoptera: Tortricidae), is not directly affected by microsporidian infection (Jones & Evenden, 2008), but infection interferes with the body size-fecundity relationship, suggesting that infected individuals cannot live up to their reproductive potential (Evenden et al., 2006). It is important to note, however, that the large aspen tortrix experiment (Jones & Evenden, 2008) was conducted using moths captured in pheromone baited traps, which might bias against sampling heavily infected individuals. In our previous work (Chapter 2), there was no statistical difference in wing area of infected and uninfected FTC moths, but the allometric relationship between wing area (mm^2) and body weight (mg) was lost. In the initial experiment (Chapter 2), FTC was inoculated with *N. disstria* spores at the third larval instar, whereas larvae were naturally infected in the current experiment. Infection later in insect development may decrease susceptibility to microsporidian infection in FTC (Wilson, 1984). Variation in infection time, however, cannot account for the lack of statistical difference in wing area between naturally infected and uninfected FTC in the 2018 experiment. Moths had relatively lower spore loads in the 2018 experiment compared to the 2014 experiment, which might have resulted in less impact of infection on adult wing size in 2018.

Wing area as measure of body size in Lepidoptera, is often correlated with fecundity (Evenden et al., 2006). Wing area can also reflect dispersal potential (Hill et al., 1999) and population quality (Hoffmann et al., 2002). It is therefore an important parameter to analyze in defoliators that undergo cyclical changes in population density, as it may help diagnose population health and outbreak stage. This is especially true for capital-breeding species that have fully developed eggs at moth emergence, like FTC (Boggs, 1981). All the nutrient acquisition occurs in the larval stage which can lead to trade-offs in resource allocation to adult life history traits (Jervis et al., 2005). For this reason, wing area is best

considered within an allometric relationship to assess resource allocation to dispersal compared to reproduction. The evolutionary trade-off between reproductive traits (ie: body mass) and dispersal potential (ie: wing area) that is depicted by the allometric relationship in FTC (Evenden et al. 2015a) could be a more informative measure of population health. Furthermore, FTC fed on a carbohydrate-biased artificial diet allocate more resources to somatic tissue than reproductive tissue (ovaries) (Colasurdo et al., 2009), suggesting diet composition plays a major role in dictating evolutionary trade-offs. Microsporidian infection of FTC (Chapter 2) altered the allometric relationship between moth body mass (mg) and wing area (mm^2). The reduced wing area observed in infected FTC in the 2014 experiment could lead to lower dispersal potential (Hill et al., 1999), as observed in other Lepidoptera (Eveleigh et al., 2007).

Microsporidian infection has other equivocal impacts on Lepidoptera wings than just size. More female spotted stem borers, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae), eclose with malformed wings after inoculation with *Nosema marucae* (Microsporidia: Nosematidae) (Ogwang & Odindo, 1993). Wing malformation seems to depend on the timing of infection. Early inoculation with *Nosema* of the sugarcane borer, *Diatraea saccharalis* Fabricius (Lepidoptera: Crambidae), in the first instar stage results in subsequent wing malformation, but inoculation at the third instar does not cause wing malformation (Simões et al., 2015). In the 2018 experiment, no significant difference was found in the proportion of insects with malformed wings between infected and uninfected FTC. Similar results have also been shown in previous findings (Chapter 2), in which the timing of the infection was delayed until third larval instar. This supports the conclusion that the presence of malformed wings in FTC is not directly related to microsporidian infection.

Larval diet influenced FTC wing size and the likelihood of wing malformation. Nutritional composition and resource availability of larval diet is known to affect resource allocation in Lepidoptera. For example, partial food depravation in the monarch butterfly, *Danaus plexippus* Linnaeus

(Lepidoptera: Nymphalidae), causes a significant reduction in wing area (Johnson et al., 2014). Diminished larval nutrition causes reduced pupal mass (Evenden et al. 2015b) and wing area in FTC and alters the allometric relationship between wing area and body weight (Chapter 2). Forest tent caterpillars also allocate more resources to somatic tissue than reproduction (ovaries) when reared on a higher carbohydrate-based diet. The opposite is true when a higher protein-based diet or a balanced carbohydrate-protein diet are used (Colasurdo et al., 2009).

Wing morphology and area can also vary with larval host and the quantity and quality of nutrition. In the 2018 experiment, FTC reared on fresh maple foliage had significantly smaller wings than larvae reared on trembling aspen. Previous studies report a large effect of larval diet on FTC fitness. For example, FTC larvae fed trembling aspen develop significantly faster, have higher fecundity and increased egg length and weight than larvae reared on sugar maple (Lorenzetti et al. 1999; Nicol et al., 1997; Trudeau et al., 2010). Moreover, FTC larvae prefer to feed on trembling aspen than sugar maple when provided a choice (Panzuto et al., 2001), which may be due to more sugar and less phenolic and tannin defense compounds in trembling aspen than sugar maple (Lorenzetti, 1993). Host affiliation appears to play a major role in dictating FTC life history traits, development time, and, potentially, population dynamics. This might explain the higher frequency of FTC outbreaks in trembling aspen than sugar maple forests in Québec (Cooke & Lorenzetti, 2006).

Moth size was differentially affected by the artificial diet in the 2014 and 2018 experiments. In 2014, moths reared on artificial diet had larger wings than the foliage-fed moths. Whereas, in 2018 FTC reared on trembling aspen had larger wings than larvae reared on sugar maple and the artificial diet. Similarly, Colasurdo et al., (2009) observed higher FTC body size when larvae were reared on fresh trembling aspen compared to larvae reared on artificial diet. Artificial diets are usually considered nutritionally superior to fresh foliage, as they are formulated to offer optimal nutritional value to insects. For example, the tobacco cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), has

increased survival and reproductive potential when reared on artificial diet compared to its natural host, the castor bean, *Ricinus communis* Linnaeus (Malpighiales: Euphorbiaceae) (Gupta et al., 2005). The Asiatic rice borer, *Chilo suppressalis* Walker (Lepidoptera: Crambidae), has faster development and heavier pupae when reared on an artificial diet compared to its preferred host, Asian rice *Oryza sativa* Linnaeus (Poales: Poaceae) (Han et al., 2012). More recently, a study on the Redbacked Cutworm, *Euxoa ochrogaster* Guenée (Lepidoptera: Noctuidae), and the Pale Western Cutworm, *Agrotis orthogonia* Morrison (Lepidoptera: Noctuidae), showed that insects develop faster and have higher pupal weight when reared on artificial diet compared to three natural hosts from different plant families (Batallas & Evenden, 2019). Foliage presents non-ingestible compounds and secondary metabolites, which may negatively affect nutrient uptake by the insect and could explain our 2014 results. The supplementation of phenolic glycosides from aspen trees to an artificial diet reduced larval development of the gypsy moths (Lindroth & Weisbrod, 1991). Artificial diet supplemented with 1% lyophilized trembling aspen leads to decreased cocoon weight, reduced wing area, higher probability of wing malformation, and delayed overall development time of FTC (Chapter 2).

The severity of microsporidian infection varied with FTC source populations. In the 2014 experiment, Alberta moths were less likely to be highly infected than moths from both Ontario populations. This could indicate that populations are in different stages of the outbreak population cycle (Anderson & May, 1980; Briggs & Godfray, 1995; Jones & Evenden, 2008; Régnière & Nealis, 2008; van Frankenhuyzen et al., 2007), as high infection levels are usually linked to high population densities (Thomson, 1960; Wilson, 1977a). Another explanation is that the microsporidia-FTC interaction varies with different populations of the host, herbivore or pathogen. Future work should focus on molecular analysis of microsporidia spores from geographically distinct FTC populations. These results would help determine the driving mechanisms which dictate the different outbreaks duration between eastern and western Canada (Cooke et al., 2012; Sippell, 1962).

This study examined a potential interaction between larval diet and microsporidian infection on adult FTC size, as measured by wing area. We also assessed the effect of diet on the expression of microsporidian infection. Moths reared on the synthetic diet were less likely to have a high spore load as adults compared to those reared on either fresh trembling aspen or sugar maple foliage. This is likely attributed to the more favourable nutritional composition of artificial diets, against the biochemical composition of foliage, therefore suggesting the presence of a cost of resistance (Janmaat & Myers, 2005). Plant foliage presents several non-ingestible compounds and secondary metabolites, which may have negatively impaired FTC susceptibility to microsporidian infection. In contrast, there was no difference in spore load in individuals reared on either fresh foliage treatment in the 2018 experiment. This finding was surprising, as trembling aspen and sugar maple are known to affect FTC susceptibility to another entomopathogen, *Bacillus thuringiensis* var *kurstaki* (Bacillales: Bacillaceae) (*Btk)* (Kouassi et al., 2001). The effect of *Btk* infection is more detrimental to FTC larvae fed on sugar maple compared to larvae reared on trembling aspen (Kouassi et al., 2001). The mechanisms behind this process remain largely unknown, however, it is likely connected to host plant biochemistry. Trembling aspen is a nutritionally superior host for FTC as it has more sugars and less phenolic compounds and tannins than sugar maple (Lorenzetti, 1993). The discrepancy between the findings by Kouassi et al., (2001) and the current study suggests that sublethal effects from high pathogen pressure, as would be experienced with a commercial application of *Btk*, may be required to reveal any subtle interactions between larval diet and infection. Other studies on FTC tritrophic interactions need to be conducted to verify this hypothesis.

Tables

Table 3-1. List of models used for statistical analyses of 2014 data. Diet (Diet = artificial standard diet, Aspen = fresh trembling aspen foliage), Origin (AB = Alberta, OD = Ontario – Dryden, OK = Ontario – Kenora), Sex (M = Males, F = Females), Infection (N = Uninfected, Y = Infected), and Infection Load (None: 0 spores, Low: 1-100 spores, High: 101+ spores count per 10 fields of view at 400x magnification).

Response	**Model**
Wing Area – Diet & Origin (Males)	glmer (Wings ~ Diet * Origin, random = (origin/eggmass)
Wing Area – Diet & Origin (Females)	glmer (Wings ~ Diet * Origin, random = (origin/eggmass)
Wing Area – Infection (Males)	glmer (Wings ~ Diet + Origin + Infection, random = (origin/eggmass)
Wing Area – Infection (Females)	glmer (Wings ~ Diet + Origin + Infection, random = (origin/eggmass)
Wing Area – Infection Load (Males)	glmer (Wings ~ Diet + Origin + Infection Load, random = (origin/eggmass)
Wing Area – Infection Load (Females)	glmer (Wings ~ Diet + Origin + Infection Load, random = (origin/eggmass)
Infection	glmer (Spore Load ~ Diet + Sex + Origin, random = (origin/eggmass)

Table 3-2. Population sizes (N) used for each analysis divided by Diet (Diet = artificial standard diet, Aspen = fresh trembling aspen foliage), Infection (N = Uninfected, Low: 1-100 spores, High: 101+ spores count per 10 fields of view at 400x magnification), and Origin (AB = Alberta, OD = Ontario – Dryden, OK = Ontario – Kenora).

	Diet		**Infection**			**Origin**		
Analysis	**Standard Diet**	**Fresh Aspen**	**Uninfected**	**Infected**		**Alberta**	**Ontario Dryden**	**Ontario Kenora**
Wing Area – Diet & Origin (Males)	285	207	*NA*	*NA*		185	223	84
Wing Area – Diet & Origin (Females)	212	156	*NA*	*NA*		172	141	55
Wing Area – Infection (Males)	185	99	167	117		87	141	56
				Low: 60	High: 57			
Wing Area – Infection Load (Females)	134	77	123	88		76	99	36
				Low: 29	High: 59			

Table 3-3. List of models used for statistical analyses of 2018 data. Diet (SD = standard diet, FA = fresh trembling aspen foliage, FM = fresh sugar maple foliage), Origin (Alberta, Ontario), Sex (M = Males, F = Females), Infection (N = Uninfected, Y = Infected), and Infection Load (None: 0 spores, Low: 1-100 spores, High: 101+ spores count per 10 fields of view at 400x magnification).

Response	**Model**
Wing Area (Males)	glmer (Wings ~ Diet + Infection, random = (Repeat)
Wing Area (Females)	glmer (Wings ~ Diet + Infection, random = (Repeat)
Wing Area (Males)	glmer (Wings ~ Diet + Infection Load, random = (Repeat)
Wing Area (Females)	glmer (Wings ~ Diet + Infection Load, random = (Repeat)
Wing Malformation	glmer (Wing Malformation ~ Diet + Infection + Sex, random = (Repeat)
Wing Malformation	glmer (Wing Malformation ~ Diet + Infection Load + Sex, random = (Repeat)
Infection	glmer (Spore Load ~ Diet + Sex, random = (Repeat)

Table 3-4. Population sizes (N) used for each analysis divided by Diet (SD = standard diet, FA = fresh trembling aspen foliage, FM = fresh sugar maple foliage) and Infection (N = Uninfected, Low: 1-100 spores, High: 101+ spores count per 10 fields of view at 400x magnification).

	Diet			**Infection**	
Analysis	**Standard Diet**	**Fresh Aspen**	**Fresh Maple**	**Uninfected**	**Infected**
Wing Area (Males)	65	75	36	93	83
					Low: 54 / High: 29
Wing Area (Females)	88	56	20	90	74
					Low: 44 / High: 30
Wing Malformation	186	152	81	218	201
					Low: 128 / High: 73

Figures

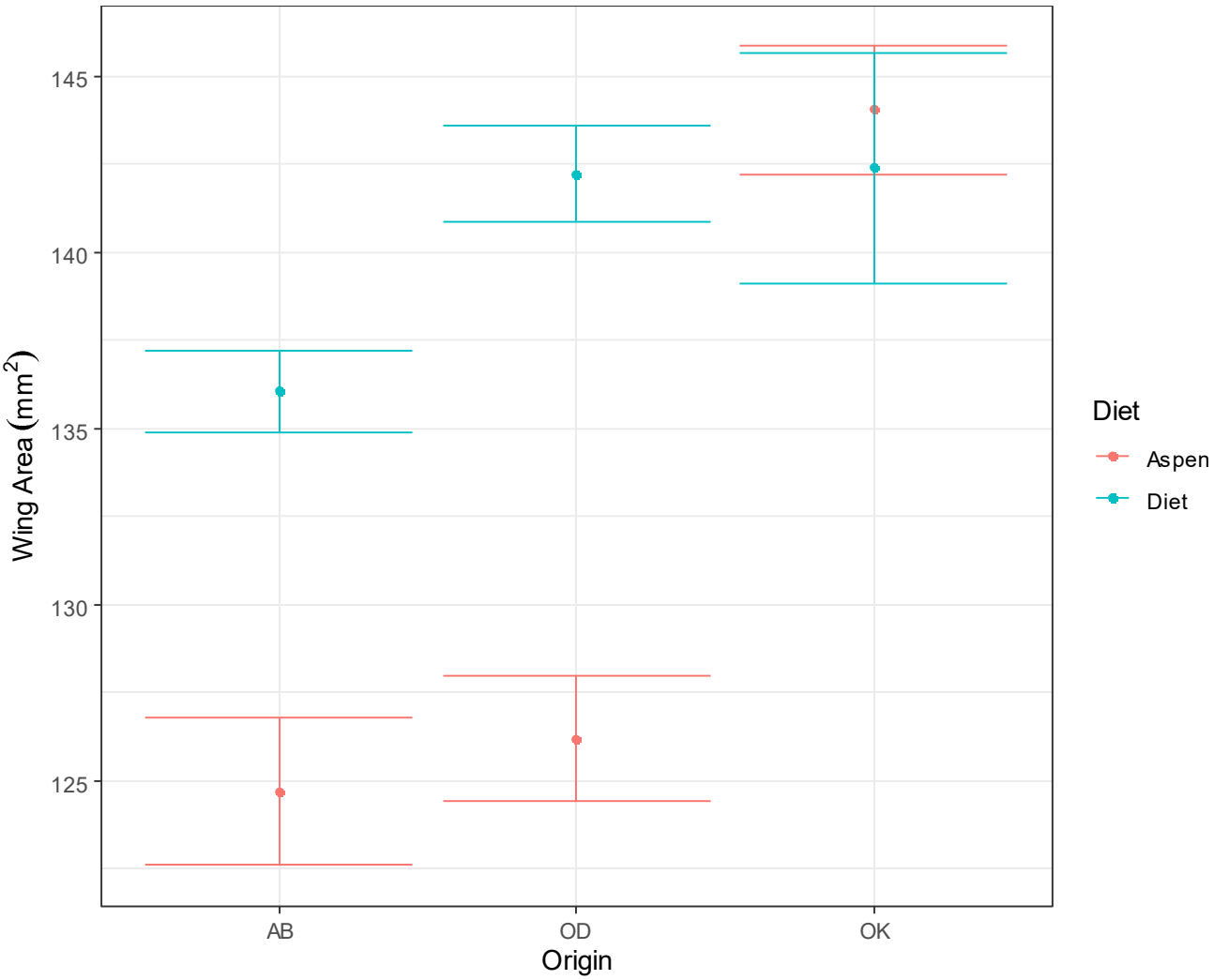

Figure 3-1. Interaction plot displaying mean wing area (mm^2) ± SE by Origin (AB = Alberta, OD = Ontario – Dryden, OK = Ontario – Kenora) and grouped by Diet (Aspen, Diet) of male FTC moths. Total moth wing area obtained by adding the areas of the right forewing and the right hindwing. Pairwise comparisons were computed with a Kenward-Roger approximation method for degrees of freedom and a Tukey p-value adjustment. AB-Diet was significantly different from AB-Aspen (t_{481} = 3.881, p = 0.002) and OD-Aspen was significantly different from OD-Diet (t_{383} = 3.864, p = 0.002). No difference was found between OK-Aspen and OK-Diet (t_{484} = 0.812, p = 0.965). To obtain moth wing area, the right forewing and the right hindwing measurements were added together. 2014 data.

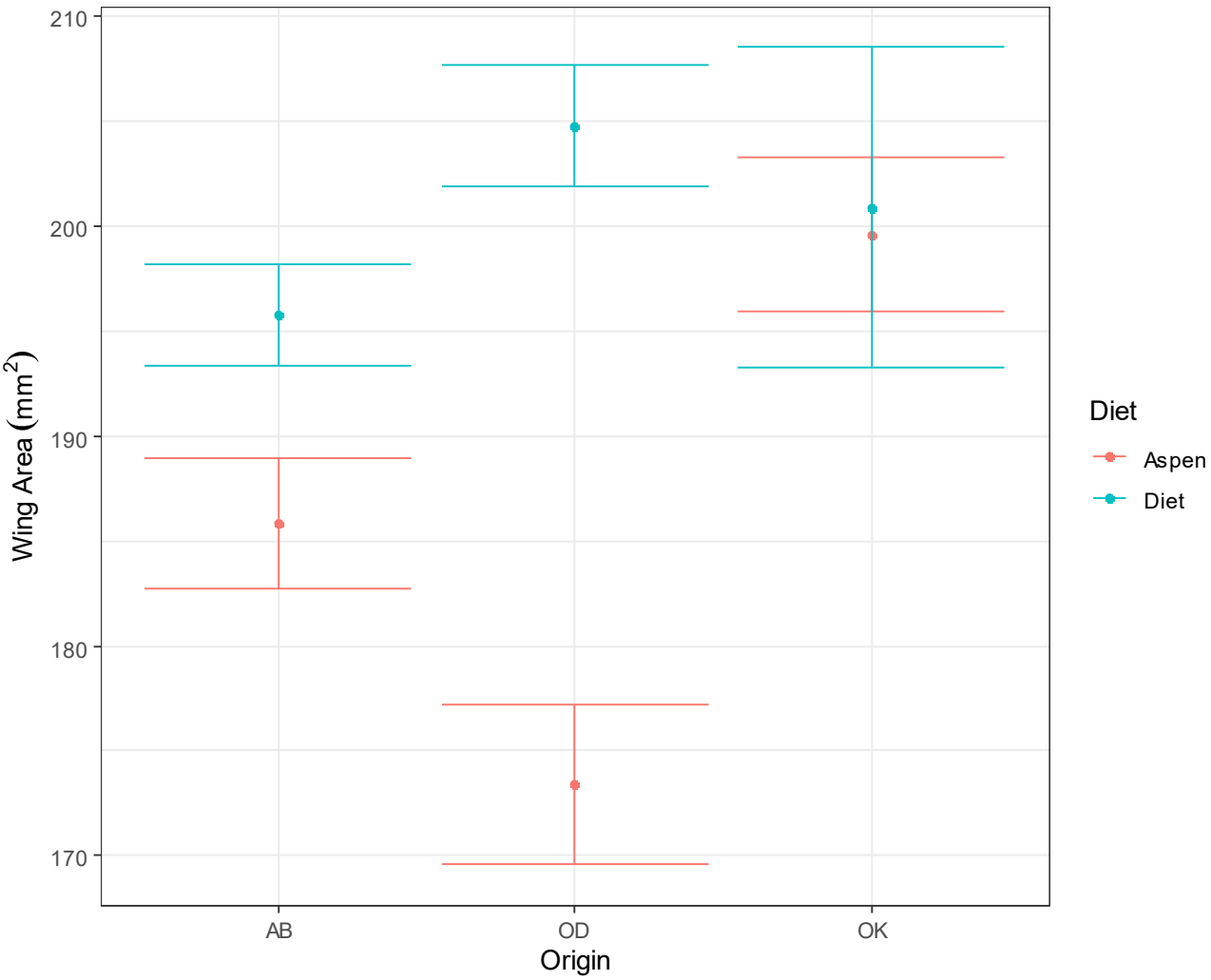

Figure 3-2. Interaction plot displaying mean wing area (mm^2) ± SE by Origin (AB = Alberta, OD = Ontario – Dryden, OK = Ontario – Kenora) and grouped by Diet (Aspen, Diet) of female FTC moths. Total moth wing area obtained by adding the areas of the right forewing and the right hindwing. Pairwise comparisons were computed with a Kenward-Roger approximation method for degrees of freedom and a Tukey p-value adjustment. AB-Diet was significantly different from AB-Aspen (t_{363}= 3.418, p = 0.009) and OD-Aspen was significantly different from OD-Diet (t_{304}= 5.035, p < 0.001). No difference was found between OK-Aspen and OK-Diet (t_{357} = 0.516, p = 0.996). To obtain moth wing area, the right forewing and the right hindwing measurements were added together. 2014 data.

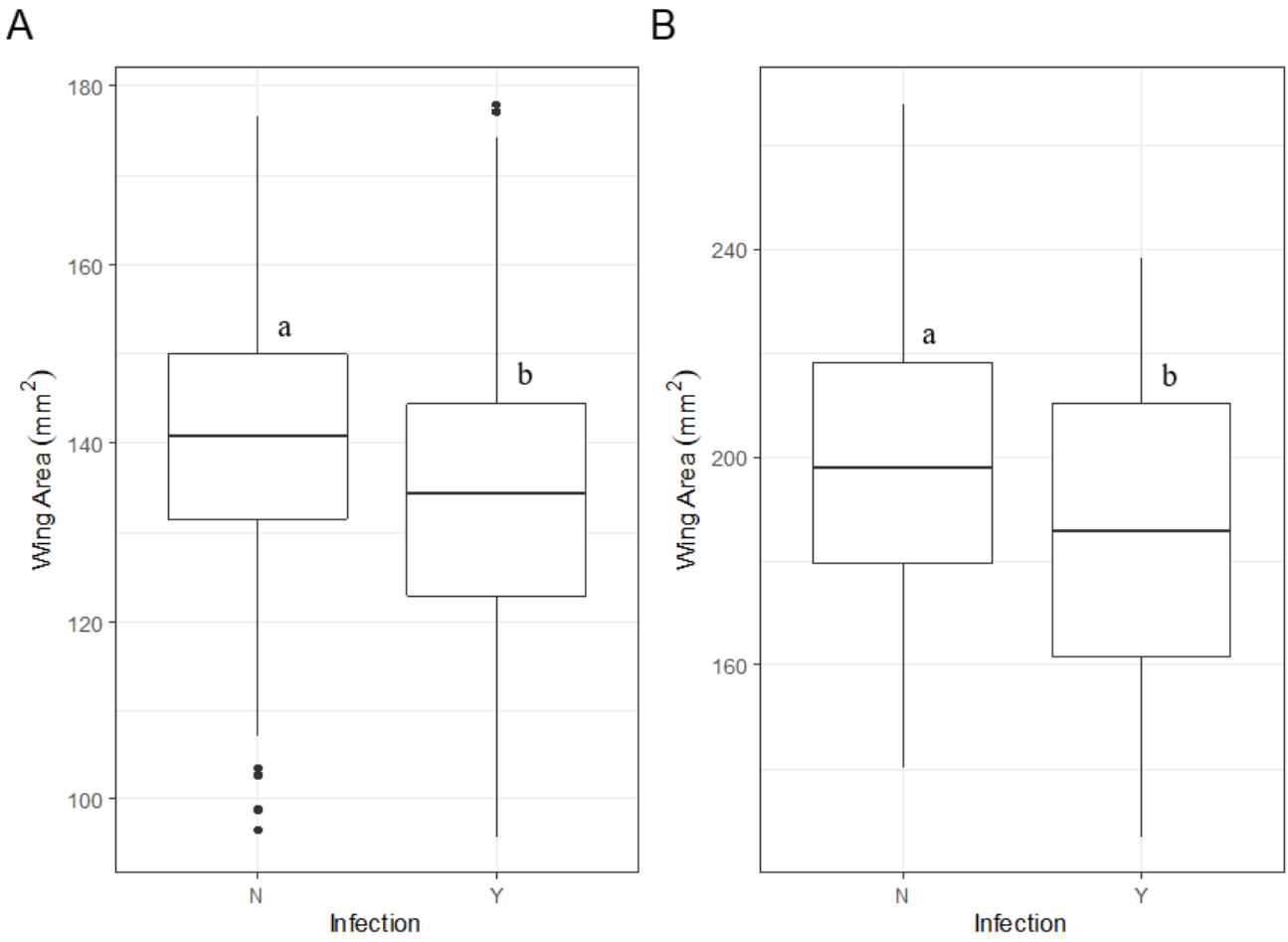

Figure 3-3. Boxplots of wing area (mm^2) of (A) male and (B) female FTC moths by Infection (N = uninfected, Y = Infected). Total moth wing area obtained by adding the areas of the right forewing and the right hindwing. The midline of each box represents the median, whereas the bottom and top of each box indicates the first and third quartiles, respectively. The whiskers extending from each box indicate the maximum and minimum values and the black dots are outliers. Boxplots marked with different letters are significantly different (F statistic, $P < 0.05$). 2014 data.

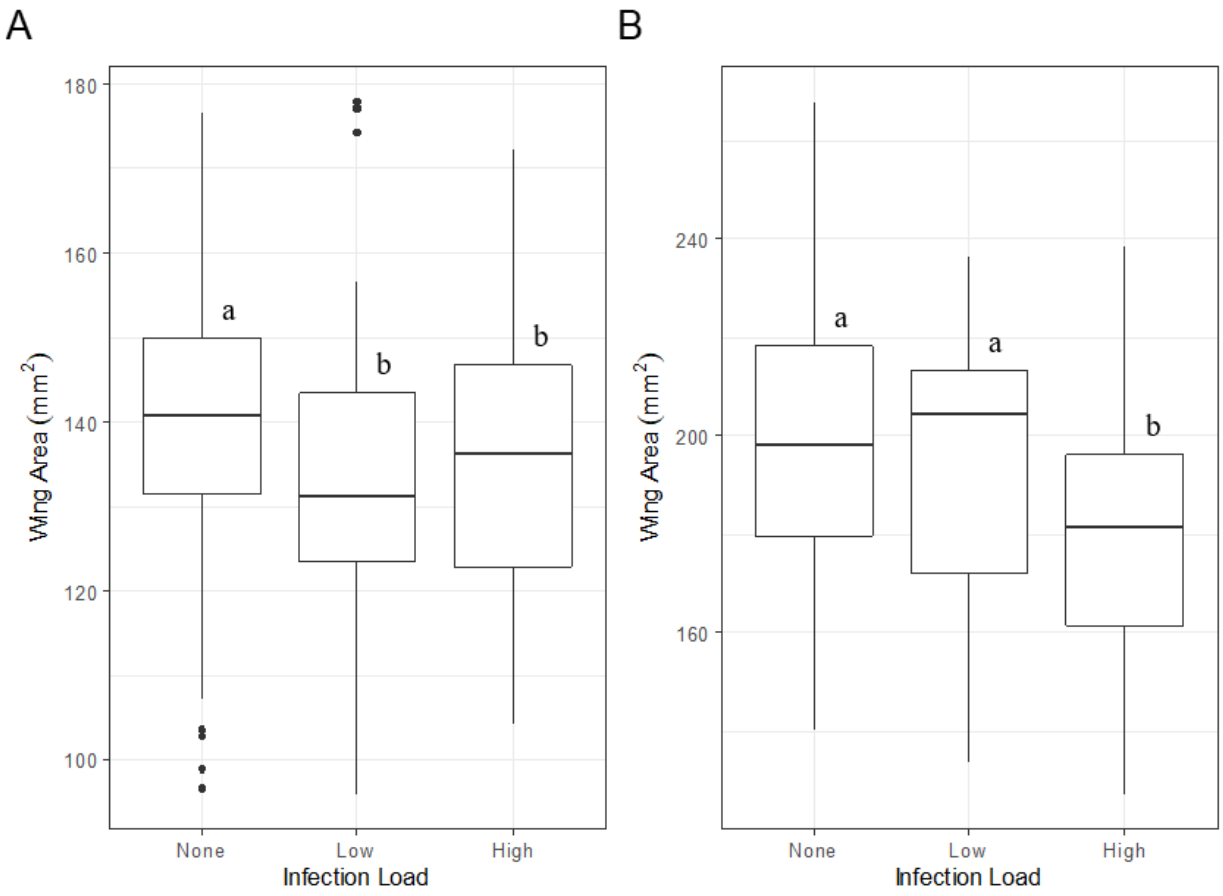

Figure 3-4. Boxplots of wing area (mm^2) of (A) male and (B) female FTC moths by Infection Load: None = 0 spores, Low = 1 < spores < 100, High = 100+ spores per 10 fields of view at 400x magnification. Total moth wing area obtained by adding the areas of the right forewing and the right hindwing. The midline of each box represents the median, whereas the bottom and top of each box indicates the first and third quartiles, respectively. The whiskers extending from each box indicate the maximum and minimum values and the black dots are outliers. Boxplots marked with different letters are significantly different (t test, P < 0.05). 2014 data.

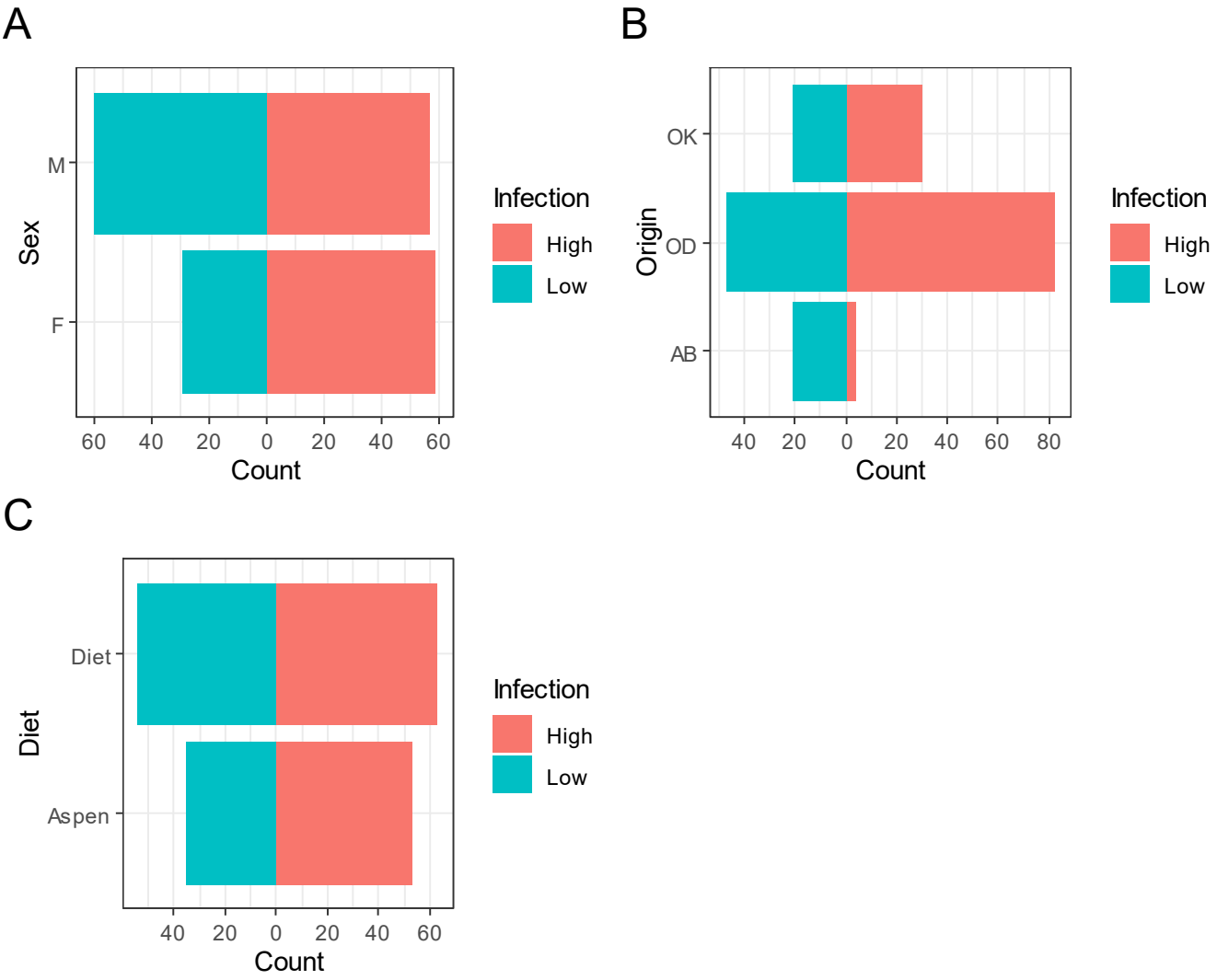

Figure 3-5. Pyramid plots of infection load (Low = 1 < spores < 100, High = 100+ spores per 10 fields of view at 400x magnification) in FTC by (A) Sex: F = Females, M = Males, (B) Origin: AB = Alberta, OD = Ontario – Dryden, OK = Ontario – Kenora, and (C) Diet: Aspen and Artificial Diet.

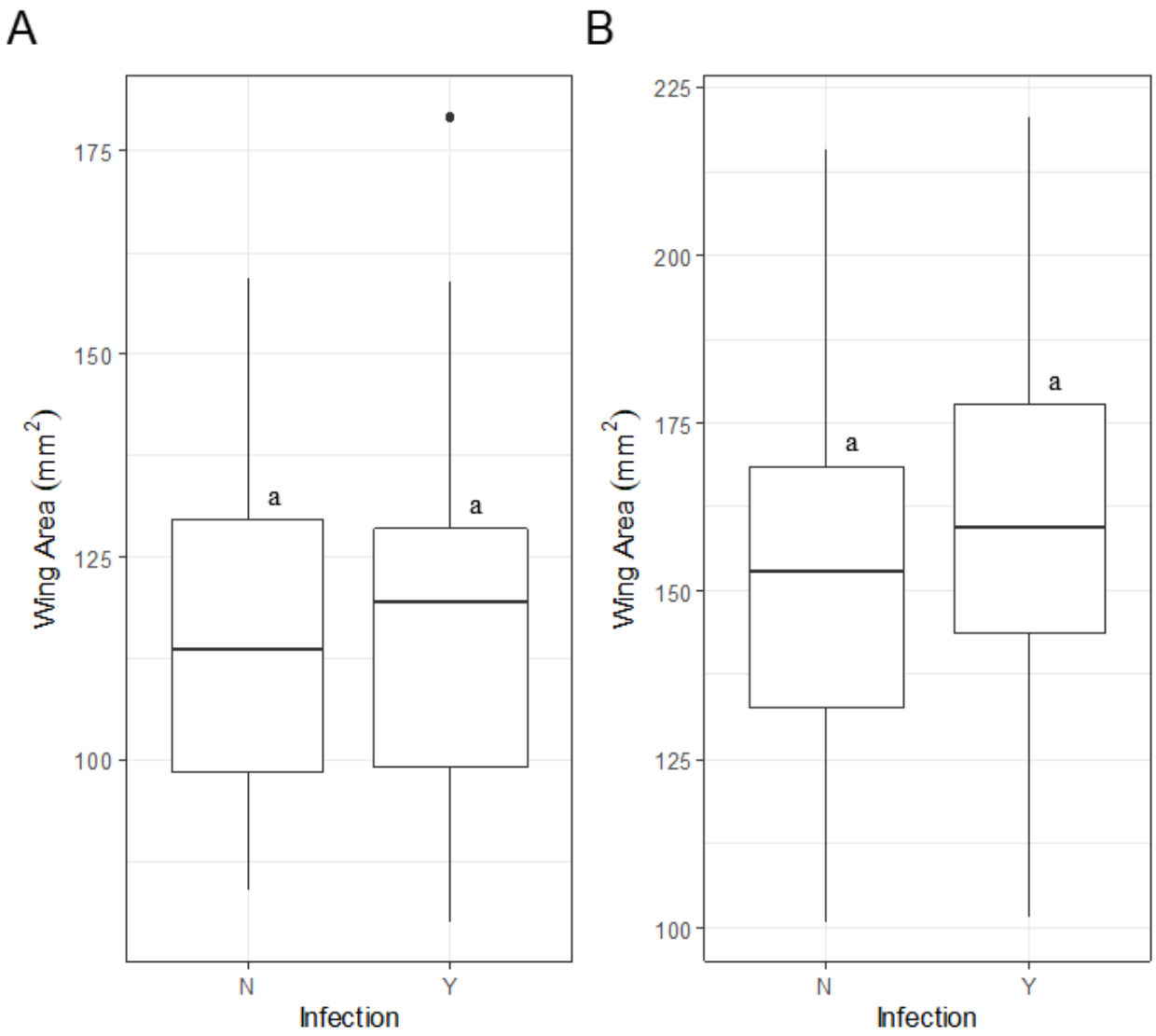

Figure 3-6. Boxplots of wing area (mm^2) of male (A) and female (B) FTC moths by Infection: Uninfected & Infected. Total moth wing area obtained by adding the areas of all four wings and dividing by two. The midline of each box represents the median, whereas the bottom and top of each box indicates the first and third quartiles, respectively. The whiskers extending from each box indicate the maximum and minimum values and the black dots are outliers. Boxplots marked with different letters are significantly different (F statistic, $P < 0.05$). 2018 data.

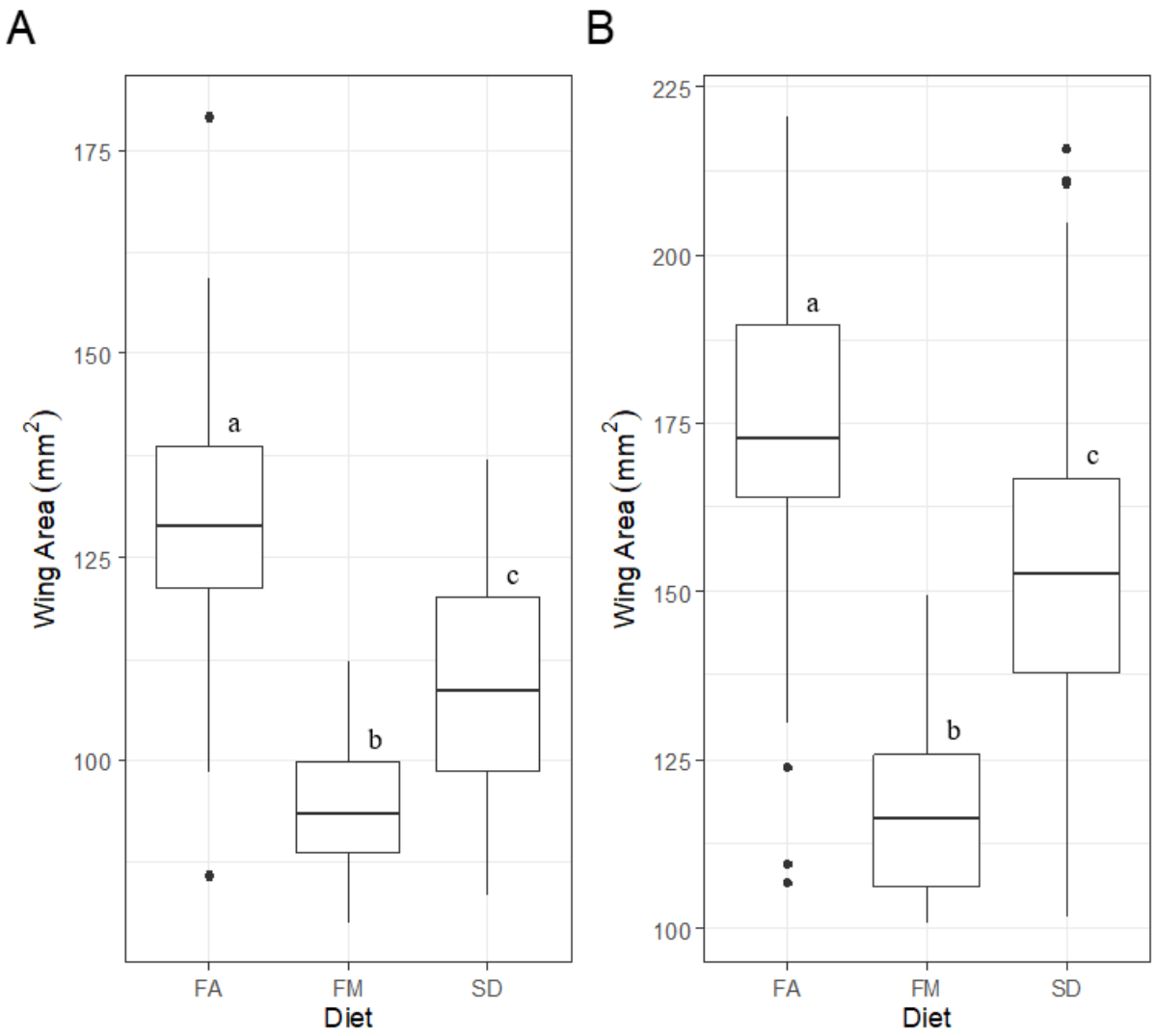

Figure 3-7. Boxplots of wing area (mm^2) of male (A) and female (B) FTC moths by diet: FA = Fresh Aspen, FM = Fresh Maple, SD = Standard Diet. Total moth wing area obtained by adding the areas of all four wings and dividing by two. The midline of each box represents the median, whereas the bottom and top of each box indicates the first and third quartiles, respectively. The whiskers extending from each box indicate the maximum and minimum values and the black dots are outliers. Boxplots marked with different letters are significantly different (t test, $P < 0.05$). 2018 data.

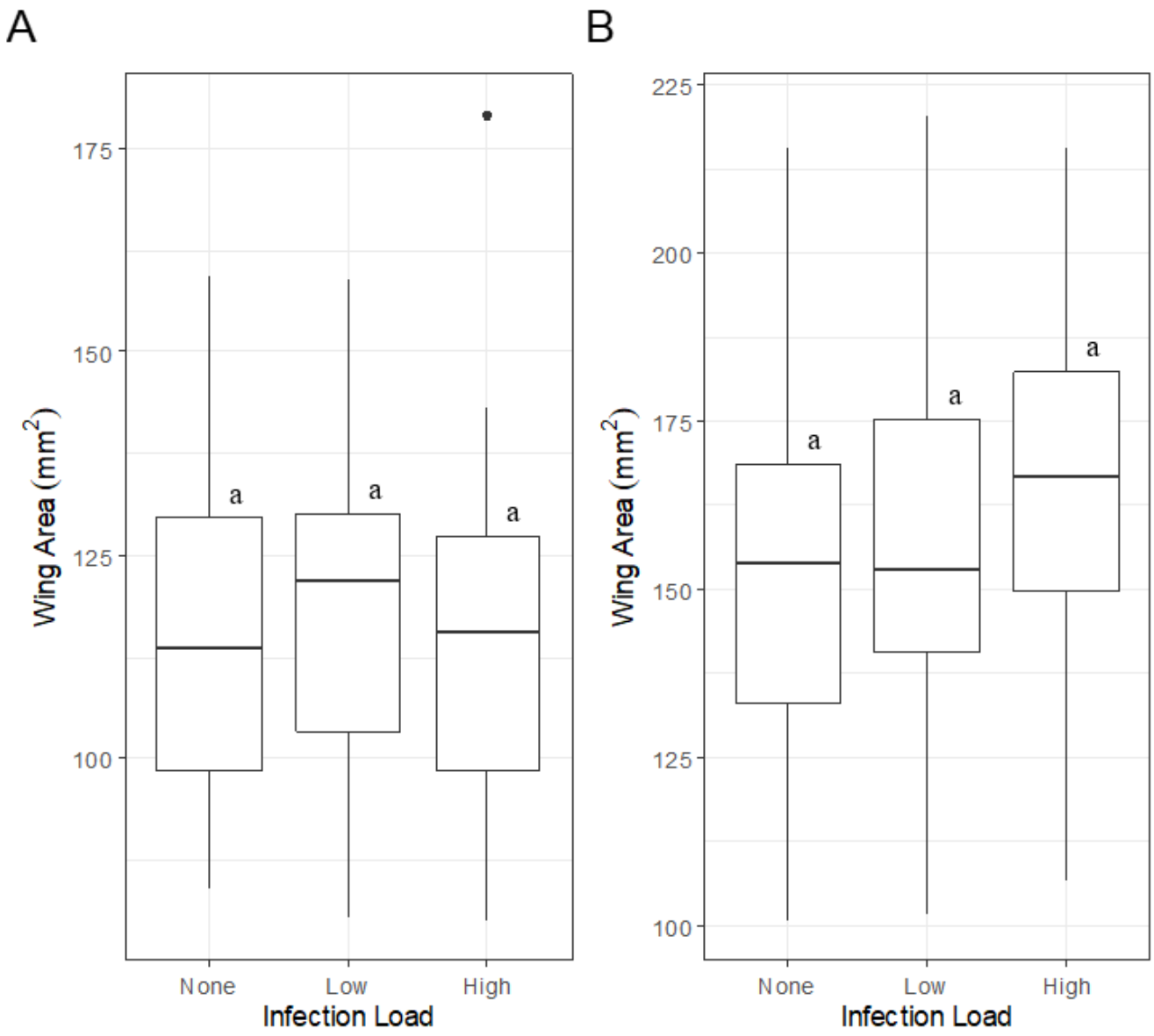

Figure 3-8. Boxplots of wing area (mm^2) of male (A) and female (B) FTC moths by infection load: None: 0 spores count, Low: 1-100 spores count, High: 101+ spores count per 10 fields of view at 400x magnification. Total moth wing area obtained by adding the areas of all four wings and dividing by two. The midline of each box represents the median, whereas the bottom and top of each box indicates the first and third quartiles, respectively. The whiskers extending from each box indicate the maximum and minimum values and the black dots are outliers. Boxplots marked with different letters are significantly different (t test, $P < 0.05$). 2018 data.

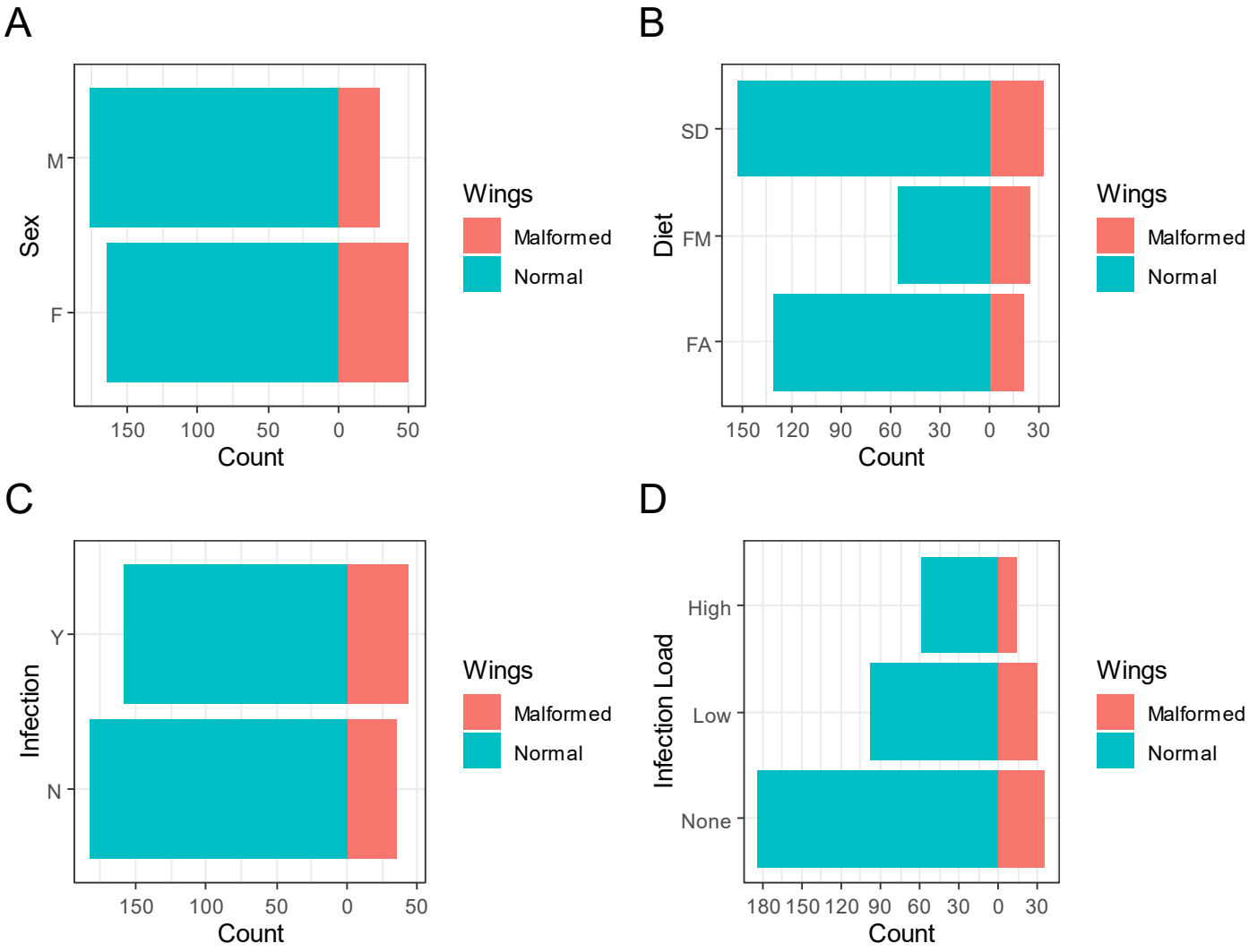

Figure 3-9. Pyramid plots of malformed wings in FTC by (A) Sex: F = Females, M = Males, (B) Diet: FA = Fresh Aspen, FM = Fresh Maple, and SD = Standard Diet, (C) Infection: N = uninfected, Y = infected, and (D) Infection Load: None: 0 spores count, Low: 1-100 spores count, High: 101+ spores count per 10 fields of view at 400x magnification. 2018 data.

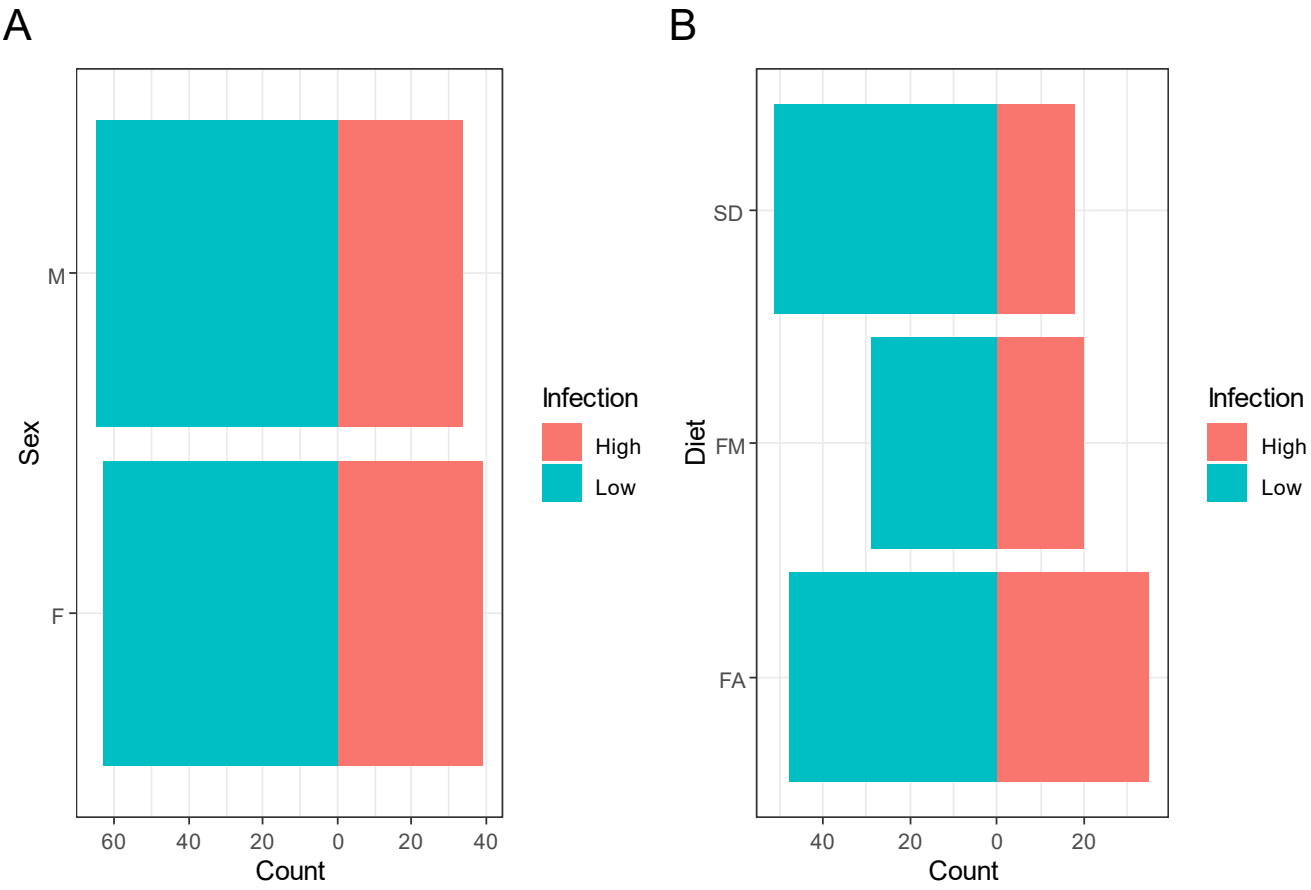

Figure 3-10. Pyramid plots of infection load (Low = 1 < spores < 100, High = 100+ spores per 10 fields of view at 400x magnification) in FTC by (A) Sex: F = Females, M = Males, and (B) Diet: FA = Fresh Aspen, FM = Fresh Maple, SD = Standard Diet. 2018 data.

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Chapter 4 – Conclusions

The forest tent caterpillar (FTC), *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae), is the most widespread tent caterpillar in North America and a major defoliator of hardwood trees (Fitzgerald, 1995; Baker 1972). This lepidopteran species is an extremely efficient forager (Fitzgerald, 1995), and larval feeding at outbreak densities can cause economic and ecological damage throughout its geographical range. Recent studies suggest FTC population cycling and outbreak dynamics may shift as result of climate change (Dukes et al., 2009; Uelmen et al., 2016; Cooke & Roland, 2018). For this reason, special attention has been given to understanding and identifying the driving forces behind its cyclical population patterns. Outbreak dynamics in FTC have been linked to numerous factors, such as forest fragmentation (Roland, 1993) and heterogeneity (Cooke & Roland, 2000), climate (Cooke & Roland, 2018), parasitism (Parry, 1995), genetics (Miller, 1996), host phytochemistry and budbreak phenology (Donaldson & Lindroth, 2008), and pathogens (Fitzgerald, 1995). The potential implication of tritrophic interactions among these factors, however, remains largely unexplored. In this thesis, I investigated the effects of larval diet on FTC susceptibility to microsporidian infection, and the potential implications of this interaction on insect life history traits.

In Chapter 2, I explored the tritrophic interaction among larval diet, FTC, and the microsporidian parasite, *Nosema disstriae* (Dissociodihaplophasida: Nosematidae), using artificial diets and direct inoculation of FTC larvae with microsporidia. With this approach, I could alter the diet quality and quantity, along with the disease status of insects. Quality of the diet was altered to explore the role of foliage phytochemistry in this tritrophic interaction via the supplementation of a 1% lyophilized trembling aspen, *Populus tremuloides* Michx (Malpighiales: Salicaceae), foliage to an artificial diet. Phytochemistry of the leaves of host trees affects FTC fitness (Jocelyn & Lindroth, 2000). Through alteration of the quantity of food provided to larvae, I could mimic the intraspecific competition that occurs at high population densities (Despland & Le Huu, 2007; Fitzgerald, 1995). In addition to larval

food source manipulation, larval disease status was modified through inoculation of larvae with standardized doses of microsporidia. Lethal and sublethal effects of microsporidian infection are factors that influence population dynamics in FTC (Fitzgerald, 1995; Wilson, 1977).

Chapter 3 complements the information gathered in Chapter 2 on this tritrophic interaction, but with a different approach. Manipulation of larval diet in Chapter 3 was conducted through provision of larvae with fresh foliage of two host species, trembling aspen and sugar maple, *Acer saccharum* Marshall (Sapindales: Sapindaceae), in comparison to artificial diet. Phytochemical differences between these two FTC host plants have been widely explored (Lorenzetti, 1993), and the implications on FTC fitness are well established (Nicol et al., 1997; Parry & Goyer, 2004; Trudeau et al., 2010). Interestingly, the foliage phytochemistry of these two species alters FTC susceptibility to *Bacillus thuringiensis* var. *kurstaki* (Bacillales: Bacillaceae), an entomopathogen used for biological control of outbreak populations of FTC (Kouassi et al., 2001). In Chapter 3, I explored the effects of naturally occurring microsporidian infection, rather than the inoculation with known spore loads used in Chapter 2. This allowed me to investigate the role of natural infection with microsporidia and its interaction with larval diet on adult moth body size, as measured using wing area.

The results of experiments conducted in both chapters of my thesis are in agreement and suggest that larval diet does not affect FTC susceptibility to microsporidian infection. This was true when the quality and quantity of an artificial diet were manipulated, as well as when different types of tree host foliage were offered to larvae. Moreover, the mode of larval microsporidian infection (natural vs inoculated) did not play a role in shaping this tritrophic interaction. Our results, however, illustrated the individual effects of microsporidian infection and larval diet on FTC development and adult life history traits. Microsporidian infection resulted in delayed FTC larval development time (Chapter 2), as observed in previous studies (Thomson, 1959; Wilson, 1977). Delayed development may increase exposure to predators, temperature fluctuations, and resource competition, and ultimately impact

fitness and/or survival of the insect. Microsporidian infection also reduced ability of FTC to produce cocoons that enclose pupae (Chapter 2). Previous research has linked microsporidian infection of FTC to reduced cocoon production (Wilson 1977), presumably due to high spore loads in the larval silk glands (Thomson 1959). Cocoons protect the vulnerable pupal stage against predators and environmental conditions (Gauthier et al., 2004; Zhao et al., 2007), and they have been linked to insect quality (Lyon & Cartar, 1996). It is not known whether high spore concentration in the silk glands (Thomson, 1959) impacts the ability of infected larvae to lay silk trails. Trails are essential for communication and foraging of colony members (Fitzgerald & Costa, 1986), a disruption of this communication system may lead to detrimental effects to FTC larvae.

Prolonged development time and absence of a protective cocoon around the pupae increase insect exposure to predators, parasites, and unfavorable environmental conditions (Gauthier et al., 2004; Zhao et al., 2007), and may also indirectly influence FTC population dynamics. It is likely that microsporidia and other biotic and abiotic factors interact to influence FTC fitness and population cycling. Feld studies that targeted gypsy moth, *Lymantria dispar* Linneaus (Lepidoptera: Erebidae) showed a 58% increase in larval parasitism in plots where *Nosema fymantriae* (Dissociodihaplophasida: Nosematidae) was sprayed (Weiser & Novotný, 1987). High microsporidian infections occur in larvae at high population densities (Fitzgerald, 1995); it is therefore possible that microsporidian infection interacts with other mortality factors to suppress populations at peak density.

Microsporidian infection in my study was associated with the complete loss of the allometric relationship between wing area (mm^2) and body weight (mg) in adult moths (Chapter 2), and a significant reduction in wing area (Chapter 3). Resource allocation is linked to an organism's life history strategy (Boggs, 1992), which may alter evolutionary trade-offs between survival and reproduction. In FTC, an allometric relationship with a steep slope indicates that body mass increases with wing area which would promote dispersal behaviours (Evenden et al. 2015). Therefore, sublethal infection with

microsporidia may alter FTC ability to disperse by flight. These observations have been made in other forest Lepidoptera (Eveleigh et al. 2007) and result from the breakdown of allometric relationships between wing area and body weight. These changes could be linked to population dynamics and may influence cyclical outbreak patterns (Hunter, 1995; Ruohomäki, 1992).

The primary and secondary metabolites in larval diet affect FTC fitness, fecundity, resource allocation, and overall population dynamics (Donaldson & Lindroth, 2008; Nicol et al., 1997; Trudeau et al., 2010). My studies, using lyophilized trembling aspen (Chapter 2) and fresh sugar maple and trembling aspen foliage (Chapter 3), illustrated that fresh maple was the least suitable diet for FTC, and resulted in the production of the smallest moths. These findings are in agreement with previous studies, which observed lower pupation rates, reduced larval and pupal weights, prolonged development time, and decreased fecundity in FTC reared on sugar maple compared to larvae reared on trembling aspen (Nicol et al., 1997; Trudeau et al., 2010). This provides further evidence that host phytochemistry directly influences FTC life history traits (Donaldson & Lindroth, 2008). Moreover, FTC reared on fresh maple foliage had a significantly lower probability of cocoon production compared to larvae reared on either fresh aspen foliage or artificial diet (Chapter 3). Cocoon production is associated with increased fitness and reduced risk of predation, parasitism, and mechanical damage (Gauthier et al., 2004; Lyon & Cartar, 1996; Zhao et al., 2007). It is therefore possible that host affiliation interacts with predation and parasitism of FTC larvae to impact survival during pupation. This suggests larval diet may also be indirectly affecting population dynamics in FTC through an interaction with other biotic and abiotic factors. Similar conclusions can be made for partially starved larvae (Chapter 2). The reduced feeding regime was applied to mimic intraspecific competition for food sources, which occurs at high population densities (Fitzgerald, 1995; Sutton & Tardif, 2007). Partially starved larvae were significantly smaller, lighter, and required a longer development time (Chapter 2). Although partial starvation and host affiliation do not interact, they may work in an additive fashion to influence population dynamics in FTC.

It is likely that host affiliation and partial starvation interact with other factors (ie: predation, parasitism, and environmental factors) to shape FTC population cycling.

In my study, plant phytochemicals were provided to larvae in different ways between experiments (lyophilized *versus* fresh foliage). Artificial diets are formulated to provide optimal nutrition to the insect and are generally nutritionally superior to foliage (eg: Batallas & Evenden, 2019; Gupta et al., 2005; Han et al., 2012). In addition, artificial diets do not contain the secondary metabolites associated with plant hosts. It was therefore surprising to observe such different results in the comparison between artificial and foliage diets between the 2014 and 2018 experiments (Chapter 3). FTC reared on fresh aspen foliage developed significantly larger wings than larvae reared on artificial diet in the 2018 experiment (Chapter 3). This has been previously observed (Colasurdo et al., 2009). In 2014, however, larvae reared on artificial diet developed into moths with larger wings compared to those reared on trembling aspen foliage in two out of the three populations tested. The different results in 2014 and 2018 may be partially attributed to the different artificial diets used in the 2 experiments. The difference in wing area between the aspen-fed and diet-fed FTC in either of the experiments in Chapter 3 was not as large as the effect of supplementation with lyophilized aspen foliage to artificial diet in Chapter 2. Addition of lyophilized trembling aspen foliage to artificial diet increased the probability of wing malformation in adult moths and resulted in an overall smaller wing area in female moths. These effects resulted in a loss of the allometric relationship between wing area (mm^2) and moth weight (mg). Addition of lyophilized aspen to artificial diet in Chapter 2 also significantly delayed larval development time. Supplementation of 1 and 10% lyophilized trembling aspen foliage to artificial diet has been linked to increased mortality and delayed development time in previous work on FTC (Despland unpublished). Lyophilized foliage contains concentrated secondary plant metabolites as the freeze-drying process does not affect phenolic compounds (Coklar et al., 2018; Garcìa et al., 2021; Meng et al., 2018; Saifullah et al., 2019). Trembling aspen contains a variety of secondary metabolites, such as

phenolic glycosides and condensed tannins (Lindroth et al., 1987; Palo, 1984). These secondary metabolites range between 1 - 8% for phenolic glycosides and 3-30% for condensed tannins in fresh foliage (Bryant et al., 1987; Lindroth & Hwang, 1996; Osier et al., 2000; Schultz et al., 1982), but their concentration has not been measured in lyophilized foliage. Phenolic compounds reduce FTC fitness (Hwang & Lindroth, 1997). From the results of Chapter 2, I can infer that supplementation of lyophilized trembling aspen to artificial diet is not a viable representative of fresh foliage. Moreover, this study provides further evidence of the impact of trembling aspen phytochemicals on FTC development and resource allocation to adult life history traits.

Overall, my study provides the first evaluation of a tritrophic interaction among microsporidian infection, larval diet, and FTC. I can conclude that host affiliation, aspen phytochemistry, and intraspecific competition for food sources do not impact FTC susceptibility to microsporidian infection. My findings, however, provide further evidence on the effect of microsporidian infection, partial starvation, and plant phytochemistry on FTC life history traits. Field applications of microsporidia targeting FTC have never been tested, likely because of limited large-scale production of microsporidia reported in previous studies (Lewis & Lynch, 1978; Wilson & Kaupp, 1975). Field studies in which infection level can be manipulated could provide valuable data on short- and long-term effects of microsporidian infection on interactions with other factors known to influence population dynamics in FTC, such as predation, parasitism, and environmental conditions. Microsporidian infection and multitrophic interactions in FTC remain a rich area of study and may help us better understand what dictates population cycling and outbreak duration.

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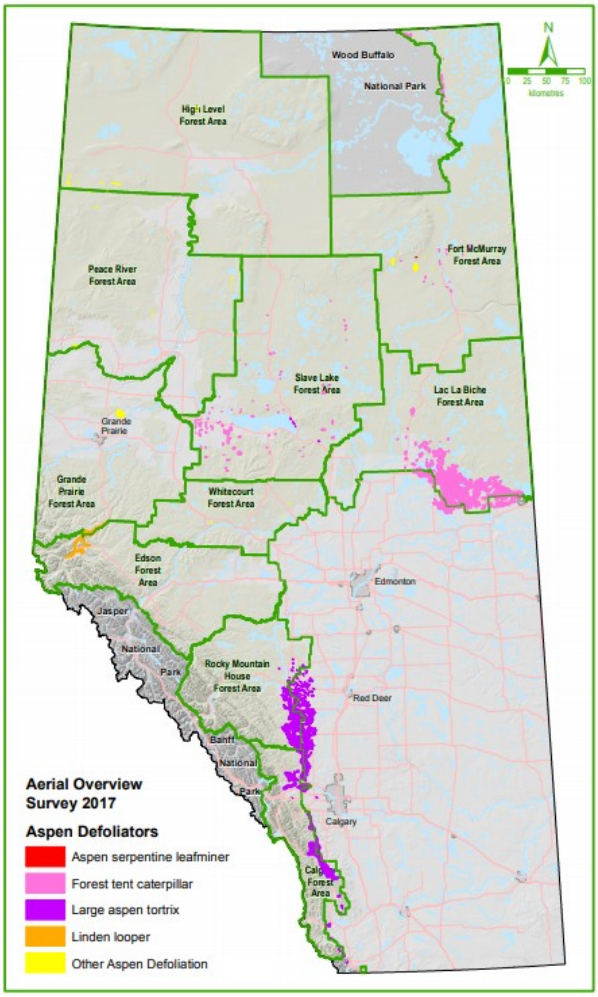
Appendix A



2017 arial survey of common trembling aspen defoliators in Alberta, Canada. Forest tent Caterpillar represented in pink. Image obtained from Alberta Government.

Appendix B

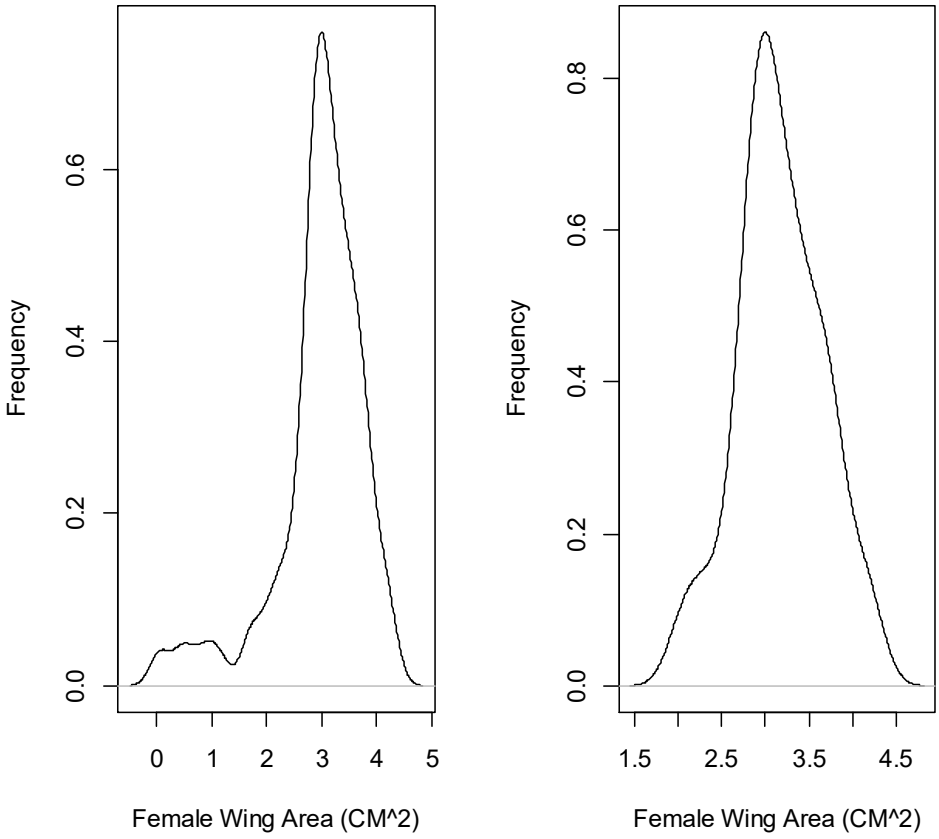
2018 Aerial overview surveys of FTC defoliation in Lac la Biche area, AB, Canada. Highlighted areas indicate high trembling aspen defoliation by FTC. Image provided by Alberta Agriculture and Forestry.

Appendix C

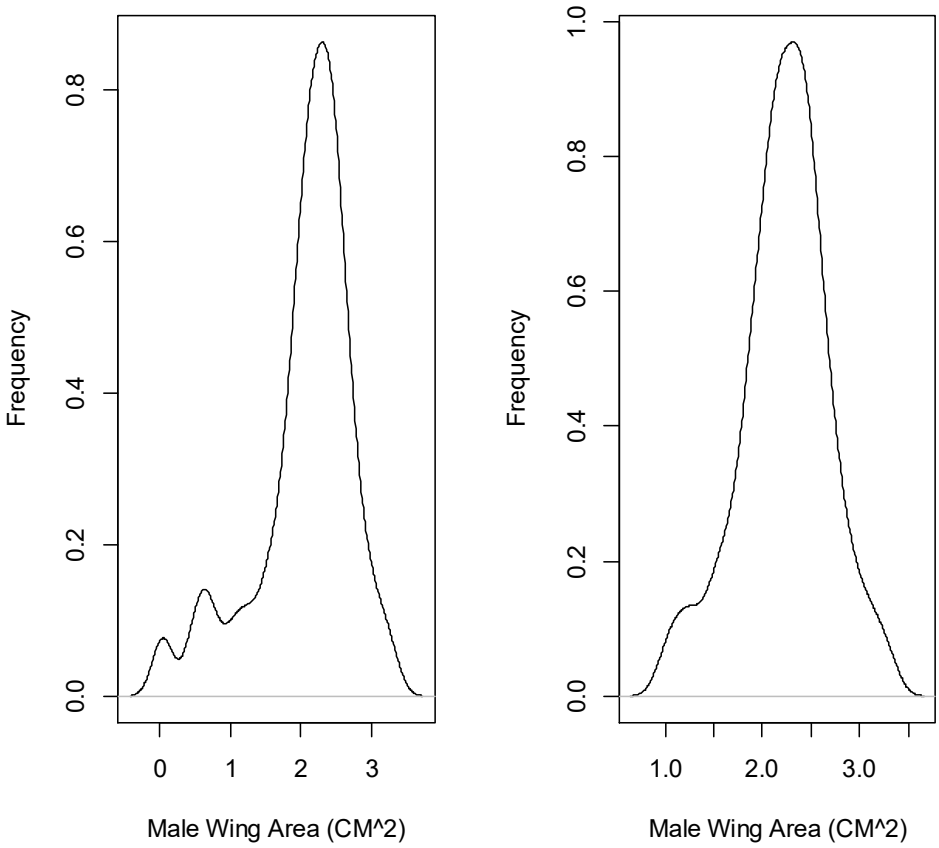
Ingredients required for the production of 1L of artificial standard diet (SD). Protocol modified from Addy (1969) diet protocol (CFS Sault Ste Marie).

Ingredients (1l)	
CASEIN	**39.2 G**
Dextrose	39.2 g
WESSON'S SALT MIX	**11.2 G**
Cholesterol	2.0 g
SORBIC ACID	**1.4 G**
Methyl paraben	0.7 g
CHOLINE CHLORIDE	**1.1 G**
Cellulose	22.0 g
RAW LINSEED OIL	**3.7 ML**
Sodium alginate	5.6 g
ASCORBIC ACID	**5.6 G**
Aureomycin antibiotic	2 g
VANDERZANT VITAMIN MIX	**16.0 G**
Wheat germ	56.0 g
AGAR	**16.8 G**
Water	200+640 ml

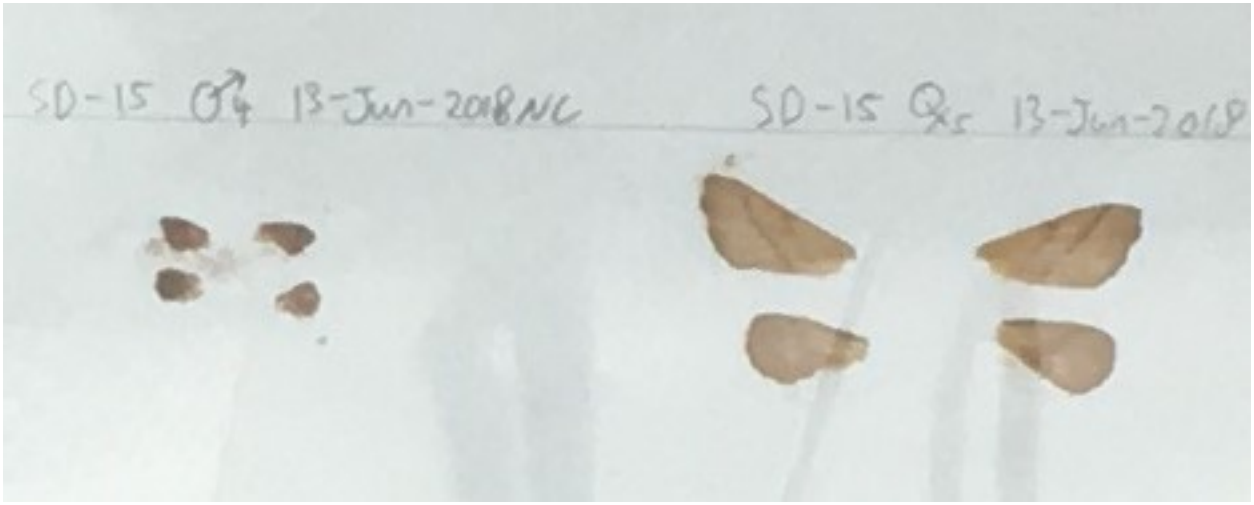
Appendix D



Frequency curve of female FTC wing area (mm^2) before (left) and after (right) manipulation. Individuals with a wing area <190mm^2 were considered "malformed" and therefore removed from "normal wings" dataset. Frequency curve was used alongside a visual inspection of wing deformation in each moth.

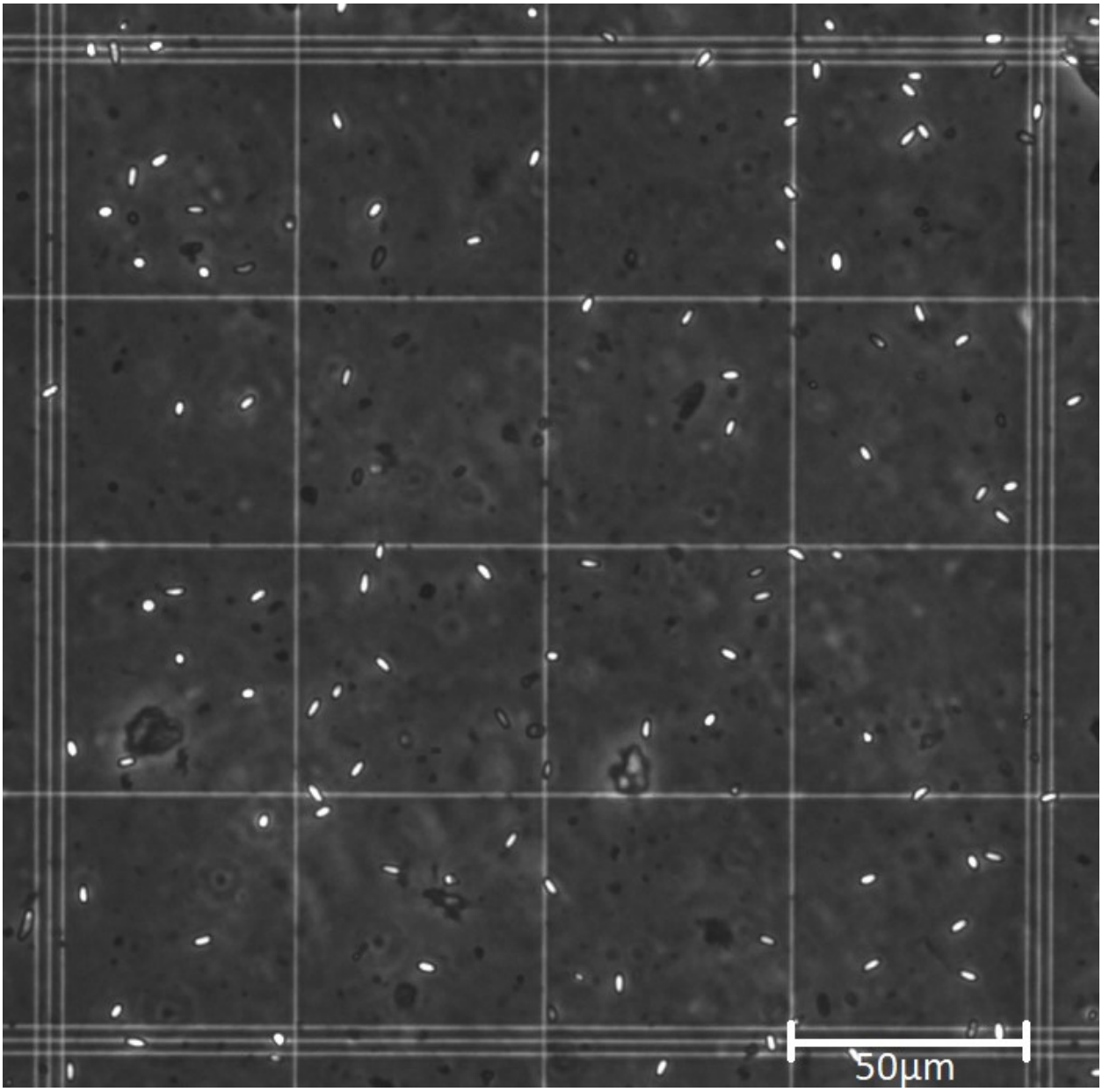


Frequency curve of male FTC wing area (mm^2) before (left) and after (right) manipulation. Individuals with a wing area <100mm^2 were considered "malformed" and therefore removed from "normal wings" dataset. Frequency curve was used alongside a visual inspection of wing deformation in each moth.



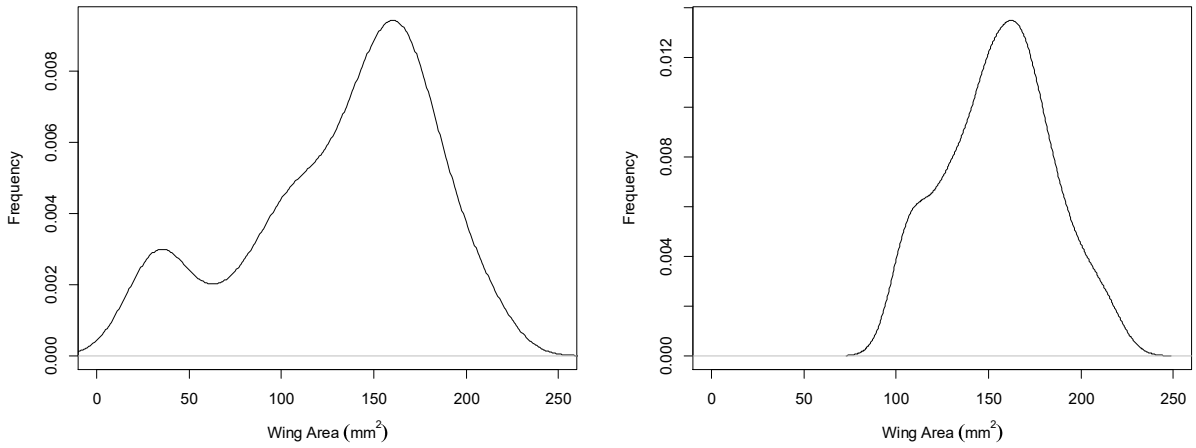
Visual example of "malformed" wings (left) compared to "normal" wings (right).

Appendix E

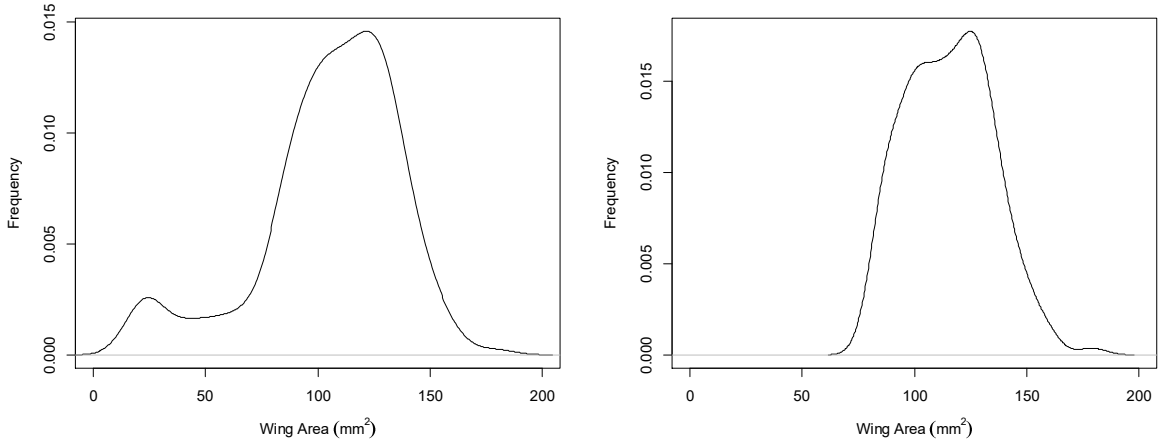


Microsporidia spores viewed with a hemocytometer at 400x magnification. Calculated size ~4.4 x 2.1 µm. Spores identified as *N. disstria*.

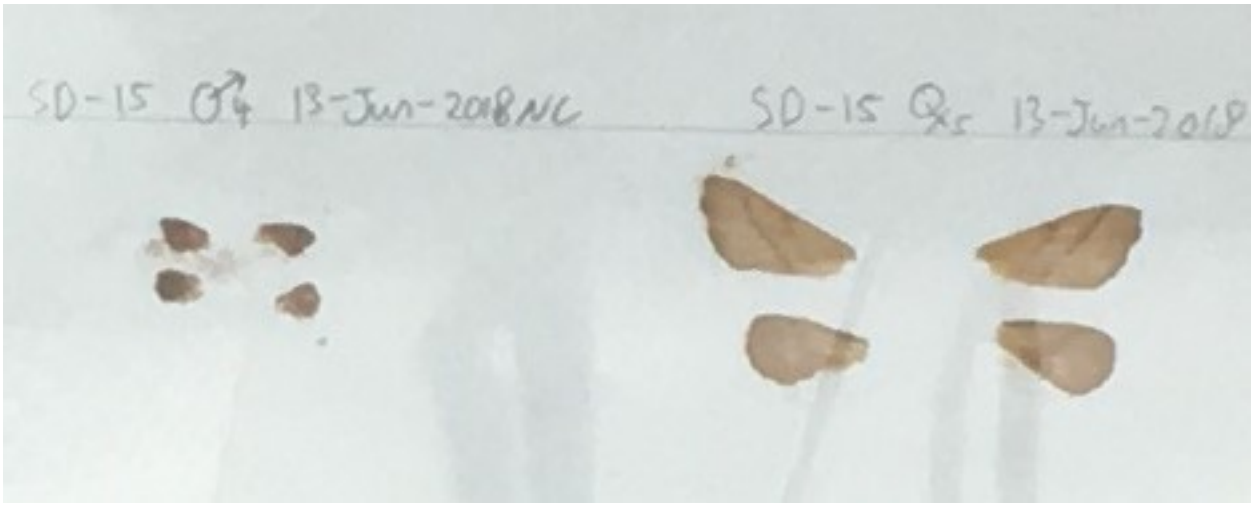
Appendix F



Frequency curve of female FTC wing area (mm^2) from 2018 before (left) and after (right) manipulation. Individuals with a wing area < 100mm^2 were considered "malformed" and therefore removed from "normal wings" dataset. Frequency curve was used alongside a visual inspection of wing deformation in each moth.



Frequency curve of male FTC wing area (mm^2) from 2018 before (left) and after (right) manipulation. Individuals with a wing area <70mm^2 were considered "malformed" and therefore removed from "normal wings" dataset. Frequency curve was used alongside a visual inspection of wing deformation in each moth.



Visual example of “malformed” wings (left) compared to “normal” wings (right).